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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

THE INFLUENCE OF CLAUDE BERNARD'S EXPERIMENTAL METHODS ON MEDICINE*

JOSEPH L. MILLER, M.D., CHICAGO, ILL.

CLAUDE BERNARD was born of humble parents at St. Julien, France, in 1813. As a boy, he apparently showed evidence of unusual ability as the curé taught him Latin. Later he was sent to a Jesuit school at Villyfranche. After completing his studies here, he went to college at Lyons, but remained only a short time. No satisfactory explanation has been given for his early discontinuance of college studies. He then entered a pharmacy in Lyons where he was taught the art of dispensing. He spent his free evenings at the theater and during this period wrote a one-act comedy, "The Rose of the Rhone," which met with considerable success. Encouraged by this, he wrote a five-act tragedy which was not a success. Apparently thinking he might have a larger field in Paris, he took his manuscript and a letter of introduction to the literary critic at the Sorbonne. The critic recognized that Bernard had considerable ability, but advised him that, inasmuch as he had worked in a pharmacy, he should study medicine and devote his spare time to literature.

In accordance with this advice, Bernard entered the Medical School of the College of France and supported himself by tutoring. Of the preclinical sciences, he was especially interested in physiology and anatomy. In dissection he became very expert as he was gifted with an unusual amount of manual dexterity. This proved to be an important factor in his later success in experimental physiology. His teachers considered him indifferent to, and indolent in, all subjects with the single exception of anatomy.

*Presidential Address Delivered before the Institute of Medicine of Chicago, December 4, 1934.

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During his fifth year he was appointed an interne and, by a happy chance, was assigned to Magendie who at this time was one of the physicians at Hotel Dieu. Magendie observed Bernard preparing an animal for experimental demonstration and was impressed by the latter's remarkable skill in dissection. He immediately appointed him his assistant in the preparation of experimental demonstrations. At this time Bernard was transferred to the College of France where Magendie held a chair.

Claude Bernard took his degree in 1843, at the age of thirty years. His thesis was on gastric juice and the part it plays in digestion.

As assistant to Magendie, he worked under the most unfavorable conditions. He had a small gloomy room in the college which served as his experimental laboratory. A small group of friends met with him on Monday evenings to discuss his experiments. The antivivisectionists were very active at this time and were responsible for many of his worries.

The following is a list of his most important discoveries:

He ascertained the method of action of the various digestive juices.

He demonstrated the rôle of the pancreas in the digestion of fats.

He showed that sugar was always present in the blood, and that it was stored in the liver and muscles in the form of glycogen.

He pointed out that when a certain spot in the medulla was punctured by a needle there followed a great increase in the blood sugar and glycosuria. This experiment was followed up to determine why this occurred, and he came to the conclusion that it was due to stimulation of sympathetic nerves.

To him belongs the credit for first demonstrating that the sympathetic nervous system was concerned with metabolism. Following this, he investigated the rôle of the sympathetic nervous system on the various organs.

He demonstrated that curare acted on the terminal ends of the motor nerves. He then investigated other poisons and found that each attacked a particular tissue. He showed that carbon monoxide entered into combination with hemoglobin. He then investigated a number of drugs and found that, like the poisons, they acted on certain tissues. He thus extended his field of experimental physiology into the domain of experimental pharmacology.

In order to appreciate Claude Bernard's influence on medicine, we should understand the condition of physiology during the first half of the nineteenth century. Vitalism, a child of Galen, and at this time the last remnant of theologic influence on science, still controlled, or at least interfered with, efforts to advance knowledge of living matter by experimental means. Vitalism was a theory (or perhaps more accurately, a creed) that there was a vital process in living matter which did not admit of explanation by mechanical or material means.

Johannes Müller, known as the father of scientific medicine in Germany, was the outstanding physiologist in his homeland at this period. In his earlier years he had made some physiologic investigations on the special senses. He was also a biologist and morphologist. He clung to vitalism, but not to the extent that he thought it impossible to explain some of nature's secrets by experimental means. He did much to encourage investigation.

In England physiology, as a profession, had few followers, but these few workers were following experimental methods.

In France the views of Cuvier, the morphologist who died in 1832, still dominated physiology. He was a through and through vitalist and consequently scoffed at experimental methods as a means of acquiring information. Bernard's chief, Magendie, was not willing to admit that vitalism was nonexistent, but he believed much could be learned from experimental studies. Michael Foster referred to him as "the apostle of experimental methods in France." He experimented, however, without previous premeditation, believing that a preconceived idea was not essential (and might be a handicap). According to Foster, "He thrust his knife here and there to see what would come out." Magendie said of himself, "I am a mere street scavenger of science. With my hook in my hand and my basket on my back, I go about the streets of science collecting what I find." (Quoted by Michael Foster in "Claude Bernard," *Masters of Medicine Series.*) However, he made many valuable contributions to science and in addition gave a great impetus to the experimental method of approach. No doubt he had a great influence on Claude Bernard.

Bernard, therefore, was beginning his life's work at a time when vitalism, which had been such a handicap to investigation, was rapidly on the wane. He had the wisdom to strike out on new lines, by combining premeditation with experimentation, and throwing vitalism into the discard. His opinion of the medicine of that day is expressed in his opening lecture to students in 1847: "Scientific medicine, gentlemen, which it ought to be my duty to teach, does not exist." Shortly after this a special chair of physiology was established for him at the Sorbonne and in 1855, after Magendie's death, he was given the latter's chair at the College of France. In 1868 he was given the highest honor that could be bestowed on a scientist, a membership in the French Academy.

In 1865 his book entitled *Introduction to the Study of Experimental Medicine* was published. This book was translated into English in 1927. This paper is largely in the form of a review of this book. Henry E. Sigerist in his book *The Great Doctor* says of this volume: "It is the fate of medical books to age quickly, but there are exceptions. If in contemporary Paris we examine the bookseller's windows in the neighborhood of the medical college, we shall find exposed for sale a work whose first edition was published in 1865." This is Claude Bernard's *Introduction to the Study of Experimental Medicine.* Sigerist refers to a statement made by Pasteur one year after this book was issued: "Never has anything clearer or more complete, more profound, been written about the true principles of the difficult art of experiment."

In his opening discussion he says that information acquired by medicine up to the present time has been through various evanescent systems, all of them empirical. He states that medicine is turning toward its permanent scientific path. Systems are being abandoned and medicine is becoming more analytical and gradually will become an experimental science. It is his opinion that the approach to this will come through applying the experimental method to physiology, to pathology, and to medicine. He continues by saying that, during the period of empiricism, physiology and medicine could advance separately, "but now they must mutually support each other with physiology as the basic science." "I think that medicine is destined to be an experimental

and progressive science and precisely because of my conviction in this respect I am putting forth this work with an object of contributing my share toward encouraging the development of scientific and experimental medicine." He discusses the difference between observation and experiment, "the former appears of itself; the latter is the fruit of an effort." He agrees with Cuvier when he says, "The observer listens to nature; the experimenter questions and forces her to unveil herself." The experimenter must have some genius as an inventor in order to devise apparatus. "In the experimental sciences all progress is measured by improvement in the means of investigation." "In scientific investigation minutiae of method are of the highest value. An instrument constructed in some special way may often suffice to solve the most abstract problem." Simple noting of facts is of limited value: "We must reason about what we have observed." An effort should be made to have some guiding lead before beginning an experiment. Occasionally an experiment is performed without any preconceived idea: "The experiment is then an observation induced with the object of bringing to birth an idea."

The experimenter must possess three qualities—he must have ideas; must be an unbiased observer; and must have manual dexterity. Bernard's early work in anatomy had trained his hand and eye. "Observers simply note the phenomena before their eyes." "Observers then must be photographers of phenomena. Their observations must accurately represent nature." "We must observe without any preconceived ideas, the mind must be passive, that is, it must hold its peace. It listens to nature and writes at her dictation."

When the experiment is begun "The experimenter must now disappear, or rather change himself instantly into an observer, and it is only after he has noted the results of his experiment exactly that his mind will come back to reason, compare, and decide whether his experimental hypothesis is verified or disproved by these results." He proceeds further along this same line: "I may say that our experimenter puts questions to nature, but as she speaks he must hold his peace, he must note her answers, hear her out, and in every case accept her decisions." "Put off your imagination, as you take off your overcoat, when you enter the laboratory, but put it on again as you do your overcoat when you leave the laboratory. Before the experiment and between whiles, let your imagination wrap you around; put it right away from you during the experiment itself lest it hinder your observing power." Bernard emphasizes this saying by another: "He must never speak for her nor listen partially to her answers by taking from the results of an experiment only those things that support or confirm his hypothesis."

An experiment is made up of two parts—premeditation on the method with which the experiment is to be carried out; and notation of the result. "I consider it, therefore, an absolute principle that experiments must always be devised in view of a preconceived idea, no matter if the idea be not clear nor very well defined." To those who condemn the use of a hypothesis or preconceived idea, he answers by saying, "They confuse the method of making the experiment with the method of recording the result." "We must give free reign to our imagination, but we must regulate it." If the hypothesis is not

verified, we at least have collected some facts that are useful to science. "The tending to a preconceived idea always has been and always will be the first flight of an investigative mind."

Bernard was not only a scientist, but also a philosopher as shown by the following quotation: "The experimental method is not a natural tendency for man. Only after lengthy wandering in theological and scholastic discussion has he recognized at last the sterility of his efforts in this direction." "The experimental mind differs from the scholastic chiefly in its modesty because his experiments make him moment by moment conscious of both his relative and absolute ignorance."

Again philosophizing, he says that the human mind in the course of evolution has passed first from feeling to reasoning and finally to the experimental method. First, feeling alone imposed itself on reason. This created the faith in theology. "Reason or philosophy, the mind's next mistress, brought to birth scholasticism." "Experimentation in natural phenomena brought forth truths concealed from feeling or reason. In the search for truth, however, feeling takes the lead: it begets the a priori idea, reason develops this idea. In turn reason must be guided by experiment." "The experimental method is concerned only in objective truths."

"Experimental ideas do not arise spontaneously. Usually such ideas arise as a result of observation." In his work he relates that not infrequently in the course of an experiment he made an observation that had nothing to do with the subject under investigation, but which furnished a lead for further experimentation.

He refers to a statement frequently made, that to make discoveries one must be ignorant. This is in line with a statement made by Sir Clifford Allbutt in regard to a famous surgeon of the sixteenth century: "He was uneducated and hence did not have the handicap of a speculative intellect." Bernard says that this statement contains a truth as uncontrolled theories lead one astray, especially if unable to ignore truths that are in conflict with one's theory. "We never make experiments to confirm our ideas but to control them."

He warns against the man who conducts his experiments only for the purpose of refuting a colleague, rather than to collect facts: "At the same time they make poor observers because they choose only those observations that suit their purpose." "A man of science wishing to find truth must keep his mind free and calm and if possible never have his lids bedewed, as Bacon says, by human passions."

If in error we must gladly admit it. "When we make a mistake, the service which we owe to truth requires that we should never fear retraction." "Let me say it is always instructive to acknowledge an error. It is not enough to say 'I was mistaken'; we must say how we were mistaken."

He discusses the conclusions that may be drawn when a positive result is followed by one or more negative ones. "Negative facts, no matter how numerous they may be, never destroy a single positive fact. That is why pure and simple negation is not criticism and this method should be absolutely rejected in science, because science is never built on negation."

To Bernard must be given the credit for recognizing the close relationship between physiology and clinical medicine. At the present time this relationship is not so widely grasped as it should be. In truth the symptoms of disease are merely the manifestations of a disturbed or pathologic physiology. We might add that the signs of disease are the expression of pathologic anatomy which in turn is responsible for the disturbed physiology.

Bernard in the third chapter of the third part of his book discusses the application of physiologic experimental methods to clinical medicine. He states repeatedly that scientific medicine will develop through a knowledge of physiology. He says that the beginning of all science is empiricism, based on a chance observation or experience. Empiricism can never be a permanent state of science, but it is the first preliminary step. While empiricism largely dominated medicine in his day, his faith was unbounded that eventually empiricism would be replaced by science through the experimental method. "The physician's new observations are generally the result of chance; that is, the patient comes to the physician and this act may be considered chance." "In such circumstances the physician's originality lies in his ability to see the fact that chance has presented to him and in not letting it escape and his only merit is accurate observation." His pride in the importance of physiology is expressed as follows: "To make good medical observation it is not only necessary to have a good observing mind, but also to be a good physiologist." "We must be, as it were, a photographer of Nature."

"All early therapeutic observations were matters of chance, the observing of the effect of certain remedies, but control experiments are necessary before drawing conclusions." He recommends the method of Pinel who said in his clinic: "This year we will observe diseases without treating them; next year we will treat them." "Only by this method are we enabled to know the course of untreated disease." "Therapeutic investigation conforms to exactly the same rules as physiological and pathological investigations." "The method of observation and experiment is still the same, unchangeable in its principles and offering only a few peculiarities in its application."

He states positively that scientific medicine does not mean that bedside observation can be dispensed with: "Quite the contrary, it is the observing physician who must be depended upon to draw conclusions." "It is essential, however, that the observer have a scientific mind; that is, he must love truth for science's sake." He criticizes those who believe that clinical medicine is largely an intuitive science—"Such physicians deny science and encourage laziness, ignorance and charlatanism." He admits that experience may be a valuable aid to the physician, largely due to accumulation of empirical knowledge, "But what I blame is wilfully staying in this empirical state and not trying to get out of it." He also criticizes those who say that medicine is largely an art: "There is no such thing as a medical artist. Physicians calling themselves such, injure medical science because they exalt a physician's personality by lowering the importance of science." He believes the best method of advancing scientific medicine is to train the medical student in experimental methods.

He refers to a statement made by Laplace, when asked why he wished to admit physicians to the Academy of Science (as medicine was not a science), "To get them among scientific men."

Referring to the origin of therapeutics: "The first tendency of man was to help his neighbor in distress. The agent employed might be a drug, or moral or religious advice." "But after this first flight of fancy which started so to speak from the heart, men have been led to reflect and, seeing the sick recover often with and without medicine, they were inclined to ask whether medicine was useful or harmful."

"Experimental medicine, unlike other cults, has no system and rejects nothing that appears reasonable, but maintains an analytical attitude toward medicine." "The scientist is not satisfied with the statement that quinine cures fever; he wishes to know the cause of the fever and the means of cure."

He says that the scientific physician is always more perplexed at the bedside than the empiricist, because he realizes the limitation of drug therapy. "He does not object to using empirical remedies but does insist on observing their effect and drawing conclusions." "Empiricism is nothing but the first step of the experimental method." "We must suffer empiricism but not accept it as a system." "Experimental medicine is only a union of expectancy and empiricism, enlightened by reason and experiment." "Medicine is destined to get away from empiricism." "Of course we shall not see scientific medicine blossoming in our day but that is not man's lot; those who sow and laboriously till the field of science are not also destined to reap the harvest." "We must remember that the one unchanging scientific principle in medicine is the absolute determinism of phenomena."

He warns against words that mean nothing, yet at first thought might appear to explain. "We must constantly be on our guard against the traps which our minds perpetually set for themselves."

He believes the teacher should simply show the student the goal: "He should then leave him free to move about and in his own way according to his own nature, only coming to his aid if he sees he is straying."

"A desire for knowledge is the moving influence in the investigator." "Those who do not know the torment of the unknown cannot have the joy of discovery which is certainly the liveliest that the mind of man can feel."

Claude Bernard's faith that advancement in medicine would, in a large measure, be due to discoveries made through experimental physiology was based largely upon the epoch-making discoveries made in his laboratory. He no doubt felt this was only a beginning effort directed toward making medicine a science. "Hence the prudent and reasonable course at the present moment is to explain all that part of disease which can be explained by physiology, and to leave that we cannot so explain to be explained by the future progress of biologic science."

The science of bacteriology at this time was unborn. However, the application of this science to medicine largely came through experimental physi-

ology. If we review the advances made in internal medicine through the past decades, we are impressed with the important rôle played by experimental physiology.

Bernard's discoveries have stood the test of time, the best evidence of the soundness of his method. I know of no more appropriate adornment for the walls of a research laboratory than some of the mottoes of this famous physiologist.

A STUDY OF THE RÔLE OF INSULIN IN METABOLISM IN NONDIABETIC PATIENTS*

I. Transitory Hyperglycemia and Glycosuria Following Discontinuation of Insulin

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THE administration of insulin to nondiabetic patients in an attempt to increase body weight dates back to shortly after the discovery of insulin by Banting and Best in 1922. Pitfield[1] in 1923 reported considerable success with its use in infantile inanition, and Falta[2] in 1925 reported uniform results in malnutrition in adults. Since then some 200, or more, reports have appeared; that of Metz[3] has been one of the most enthusiastic. Generally most of the cases reported have shown some gain in weight. However, in this large number of publications data aside from the essentially "clinical" are strikingly lacking, even blood sugars. The administration of insulin to nondiabetic subjects has apparently been regarded as a physiologic procedure presumably not associated with any evidence of a disturbance of metabolism or "deleterious effects."

This laboratory became interested in this subject upon observing a rise in the blood sugar following discontinuation of insulin given to an anorexia case, which suggested that a temporary hyperglycemia and even a glycosuria might be induced in this way in nondiabetic subjects. Data supporting this suggestion obtained from blood sugars and glucose tolerance tests in a number of patients during and following a period of insulinization have been presented in a preliminary report (Paul, Clark, and Gibson 1932).[4] These experiments are reported in this paper in detail. Independently Wilder, Smith, and Sandiford[5] observed a decrease in glucose tolerance in two obese patients in whom they were studying the effect of insulin upon the rate of weight loss.

METHOD

Usually after a period of hospitilization, during which blood sugars,[6] 50 gm. glucose tolerance tests,[7] and body weight were observed, insulin was

*From the Laboratory of Pathological Chemistry, and the Department of Internal Medicine, the State University of Iowa.
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started with 5 units thirty minutes before each of the three meals. At intervals of several days as indicated by the blood sugar two hours after breakfast, the insulin dosage was increased a few units at a time until one patient was receiving as much as 75 units daily. All of the patients in this series were on a general diet with one exception, Case I. N., who was on a high carbohydrate diet. Blood sugar and qualitative urine sugar and acetone determinations were made daily in most instances. After the insulin dosage had been increased to 36 to 75 units daily and maintained at this level for several days, the insulin injections were suddenly discontinued. Blood sugar and urine sugar determinations were made after each meal until two-hour postabsorptive blood sugar level had returned to normal (pre-insulin level), and the glycosuria had disappeared. Glucose tolerance tests were observed in two cases at the time of discontinuation of insulin, and in one other case both during the course of insulinization and at intervals after its interruption. When tolerance tests were performed during a period of insulinization, insulin and breakfast were omitted on the morning of the test, the patient was given 50 gm. of glucose by mouth, and then the regular dosage of insulin was resumed with the noon meal.

SUMMARY OF RESULTS

The detailed account of these experiments is given in the accompanying case reports and tables.

During the periods of insulinization when insulin was given in progressively increasing amounts to as high as 75 units daily, blood sugars two hours after breakfast were usually normal, sometimes somewhat elevated. There was a tendency, however, in some of the subjects for the afternoon blood sugar to be somewhat low; in such cases a malted milk was allowed in the afternoon in addition to the usual diet. Hypoglycemic symptoms were rarely observed. Only one hypoglycemic shock was encountered, Case E. L., following vomiting of his supper; this, however, was promptly relieved by an intravenous injection of glucose.

After the first meal following discontinuation of the insulin injections, a certain degree of hyperglycemia was uniformly observed, the blood sugar rising as high as 250 to 300 mg. in some instances. Four of the six patients showed some glycosuria. Readjustment to the pre-insulin blood sugar level was prompt in some cases requiring only one or two days; in other subjects as long as three days or more were required before the hyperglycemia subsided and the glycosuria disappeared. Glucose tolerances in three cases were diminished at the time the insulin injections were interrupted. One of these, Case H. J., showed a progressive diminution of glucose tolerance with increasing amounts of insulin; at the time insulin was discontinued when the patient was receiving insulin units 25 t. i. d. or 75 units daily, the curve was of the mildly diabetic type, reaching a maximum of 253 mg. at the end of 1.5 hours. The return of the tolerance test to normal in this patient was slow; even after eight days it had not returned to the original level.

In connection with a study of the respiratory quotients, four other patients have been given insulin in doses varying from 33 to 81 units daily. All

of these subjects showed some degree of hyperglycemia when the insulin was discontinued, and two showed a glycosuria. The glycosuria in one patient persisted for five days.

A gain in weight has been observed during the period of insulinization in all of the patients except two to whom we have given insulin; the gains amounting to from four to eighteen pounds during periods of two to six weeks. These patients were kept on a roughly constant diet, except for an afternoon lunch in two cases; the increase in appetite was not marked.

CASE REPORTS

M. G., female, aged fifty-nine years, was admitted to the University Hospital Nov. 19, 1931. Her entrance complaints were insomnia, anorexia, and loss of weight of one year's duration. The physical examination and laboratory findings were normal. The patient's weight on admission was 115 pounds. She was started on insulin November 29 with 5 units thirty minutes before meals; on December 5 the dosage was increased to 10 units before breakfast, and 5 units before lunch and supper. It was observed that the insulin caused little decrease in the two-hour postabsorptive blood sugar level. Insulin was discontinued on December 9 with the evening injection. The blood sugar on the following morning was

TABLE I

M. G. HOSPITAL NO. F9881

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT
	mg. per cent	units	pounds
11/25/31	170		
11/29		0-5-5	113
11/30		5-5-5	
12/ 1		5-5-5	119
12/ 2	186	5-5-5	
12/ 3	133*	5-5-5	
12/ 4	159	5-5-5	
12/ 5	118	10-5-5	120
12/ 6	152	10-5-5	
12/ 7	189	10-5-5	
12/ 8	98	10-5-5	122
12/ 9	124	10-5-5	
12/10	226		
12/11	94		
12/12	133		122
12/14	124		

*Blood sugar taken at 2 P.M. All other blood sugars are at 9 A.M. two hours following breakfast.

226 mg. which was significantly higher than any value previously obtained. The blood sugars on subsequent mornings were only slightly elevated above normal. During the period of insulinization, the patient's weight had increased to 122 pounds, a gain of nine pounds.

E. L., male, aged seventy years, was admitted to the hospital April 27, 1932. His entrance complaint was gastric distress relieved by vomiting. He stated that the vomiting was usually induced by irritating his pharynx. The positive physical findings were marked weight loss and tenderness over lumbar sacral spine. Laboratory findings were normal. On roentgenologic examination, the stomach and duodenum were normal, but osteoarthritic changes were found involving the lumbar sacral spine. The patient's weight was 111 pounds on admission and increased to 115 pounds during the first five days. Insulin was started on the seventh day, May 4, with 5 units before meals, increased to 7 units t. i. d. on May 5, 10 units on May 12, and 12 units on May 17. During this period morning blood sugars were normal, sometimes somewhat elevated; however, some of the afternoon blood sugars were as low as

TABLE II

E. L. HOSPITAL No. G3821

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
5/ 2/32			115	
5/ 3	100			
5/ 4	133 (2 P.M.)	5- 5- 5		
5/ 5	44 (2 P.M.)	5- 7- 7		
5/ 6		7- 7- 7		
5/ 7		7- 7- 7	117	
5/ 8	173	7- 7- 7		
5/ 9	50 (2 P.M.)	7- 7- 7		
5/10	30 (2 P.M.)	7- 7- 7	117½	
5/11	100	7- 7- 7		
5/12	189	7-10-10		
5/13	112	10-10-10		
5/14	118	10-10-10	118	
5/15	102	10-10-10		
5/16		10-10-10		
5/17		10-10-12	120	
5/18		12-12-12		
5/19	78 (2 P.M.)	12-12-12		
5/20		12-12-12		
5/21	120	12-12-12	121½	
5/22	28 (8 P.M.)*	12-15-15		
5/23	179	10-12-12		
5/24	259 (2 P.M.)	12- 0- 0	122½	Glycosuria 1.1%
	176 (7 P.M.)			
5/25	167			Glycosuria trace
	145 (2 P.M.)			Glycosuria trace
5/26	159			Glycosuria trace
	104 (2 P.M.)			
5/27	147			

*Hypoglycemia shock following vomiting of supper. Intravenous glucose was given.
Blood sugars were taken at 9 A.M., two hours after breakfast, unless otherwise indicated.

30 or 50 mg. without definite symptoms of hypoglycemia. To obviate this possibility, the patient was allowed a malted milk in the afternoon. On the evening of May 22, he had vomited his supper without reporting it to the nurse. At 8 P.M. he was found in hypoglycemic shock with a blood sugar of 28 mg. A few grams of glucose intravenously promptly relieved the symptoms. Insulin was discontinued on May 24 with the morning injection as the last. The blood sugar two hours after breakfast was 179 mg., but after the noon meal without insulin, the blood sugar was 259 mg. with a glycosuria of 1.1 per cent. The blood sugar at 7 P.M. was 176 mg. with a trace of sugar in the urine. Blood sugars on the two following days were 167 mg. and 159 mg., respectively, with still a trace of sugar in the urine. By the fourth day after discontinuing insulin, the morning blood sugar had dropped to 147 mg. and the urine gave no qualitative test for sugar. The patient gained seven pounds during the period of insulinization.

I. N., male, aged forty-nine years, was admitted to the hospital April 24, 1932. A diagnosis of arteriosclerosis, duodenal ulcer, and incomplete heart block was made. The patient was placed on a high carbohydrate diet. His weight on admission was 123 pounds, but at the beginning of the experiment, May 4, was 120 pounds. Insulinization was started on May 4 with 5 units before meals and increased the following day to 7 units t. i. d. The dosage was later increased to 10, 12, and 15 units before meals on May 12, 17, and 22, respectively. Blood sugars both morning and afternoon were normal or somewhat elevated. Insulin was discontinued with the evening injection on May 24 as the last. The blood sugar at 9 A.M. the following day was 253 mg. with a glycosuria of 0.5 per cent, and at 2 P.M. 179 mg. By the next day the blood sugar level had returned to normal. The patient's

TABLE III

I. N. HOSPITAL NO. G582

DATE	BLOOD SUGAR 9 A.M.	BLOOD SUGAR 2 P.M.	INSULIN BEFORE MEALS	BODY WEIGHT
	mg. per cent		units	pounds
5/ 3/32	120			120
5/ 4		140	5- 5- 7	120
5/ 5		92	7- 7- 7	120
5/ 6		122	7- 7- 7	120
5/ 7			7- 7- 7	119
5/ 8	140		7- 7- 7	121
5/ 9		104	7- 7- 7	121
5/10		110	7- 7- 7	120
5/11	85		7- 7- 7	120
5/12	182		7-10-10	120
5/13	95		10-10-10	120
5/14	98		10-10-10	120
5/15	122		10-10-10	120
5/16			10-10-10	120
5/17	114		10-10-12	121
5/18			12-12-12	121
5/19		138	12-12-12	122
5/20			12-12-12	122
5/21	147		12-12-12	122
5/22			12-15-15	125
5/23	78		15-15-15	125
5/24			15-15-15	124
5/25	253*	179		125
5/26	124	140		126
5/27	98			

*Glycosuria 0.5 per cent.

TABLE IV

E. H. HOSPITAL NO. G4989

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
6/18/32	109*, [1]	0- 0- 5	101½	*Fasting; tolerance
6/19	118	5- 5- 7		
6/20	104	7-10-10		
6/21	95	10-10-10	102¾	
6/22	104	10-12-12		
6/23	59	12-12-12		
6/24	81	12-13-13		
6/25	84	13-14-14	101½	
6/26	66	14-14-14		
6/27	98	14-14-14		
6/28	110	14-14-14	100½	
6/29	79	14-14-14		
6/30	105	14-14-14		
7/ 1	96	14-15-15	101¼	
7/ 2	82	15-15-15		
7/ 3	67	15-15-15		
7/ 4	110	15-15-15		
7/ 5	87	15-15-15	100	
7/ 6	100	15-15-15		
7/ 7	99	15-15-15		95 at 2 P.M.
7/ 8	94	15-15-15		
7/ 9	104*, [2]			*Fasting; tolerance
7/10	100			
7/12			100	

*Glucose tolerance: 50 gm. glucose by mouth

Hours	0	0.5	1	1.5	2	Urine
[1]B. S. mg. per cent	109	112	135	140	112	Negative
[2]B. S. mg. per cent	104	157	192	109	82	Negative

Blood sugars were ordinarily taken at 9 A.M., two hours after breakfast.

TABLE V

E. G. HOSPITAL NO. G2413

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
6/ 7/32			108	
6/ 9	92*, [1]			*Fasting; tolerance
6/10		0- 5- 5		
6/11	112 (2 P.M.)	5- 5- 5		
6/12	118 (2 P.M.)	5- 5- 6		
6/13	78 (2 P.M.)	6- 6- 6		
6/14	85 (2 P.M.)	6- 6- 6		
6/15	133 (2 P.M.)	6- 6- 8		
6/16	135 (9 A.M.)	8- 8-10		
6/17	114 (2 P.M.)	10-10-10		
6/18	99 (9 A.M.)	10-10-10		
6/19	116 (9 A.M.)	10-10-12		
6/20	79 (2 P.M.)	12-12-15		
6/21	52 (9 A.M.)	15-15-15		
6/22	90 (2 P.M.)	15-15-15		
6/23	39 (9 A.M.)	15-15-15		
6/24	65 (9 A.M.)	15-15-15		
6/25	162 (9 A.M.)	15-15-18		
6/27	60 (9 A.M.)	18-18-18		
6/28	57 (9 A.M.)	18-18-18	112	
6/29	100 (9 A.M.)	18-18-18		
6/30	69 (9 A.M.)	18-18- 0		
	120 (2 P.M.)			
	295 (7 P.M.)			Glycosuria 0.139
7/ 1	133*, [2]			*Fasting; tolerance
	159 (7 P.M.)			
7/ 2	138 (9 A.M.)			
7/ 3	124 (9 A.M.)			
7/ 4	179 (9 A.M.)			
7/ 5	129			
7/ 7			114	

*Glucose tolerance: 50 gm. glucose by mouth

Hours	0	0.5	1	1.5	2	3	Urine
[1]B. S. mg. per cent	92	172	226	208	176		Negative
[2]B. S. mg. per cent	133	200	182	236	241	142	1 hr. 0.54%
							2 hr. 0.22%

weight had increased to 124 pounds at the time insulin was stopped. It is interesting to note that the insulin had no effect on the cardiac lesion and no strikingly favorable effect on his gastric distress.

E. H., female, aged twenty-two years, was admitted to the hospital June 2, 1932. A diagnosis of amebic dysentery and ulcerative colitis was made. The patient was poorly nourished; her weight on admission was 101 pounds. Insulinization was started on June 18, with 5 units before meals, and subsequently increased a few units at a time at intervals of a few days, until the patient was receiving 15 units t. i. d. on July 1. Blood sugars during the entire course were usually normal, sometimes somewhat lower. A glucose tolerance test was done before and immediately after the period of insulinization. The curve of the pre-insulin period was normal showing a maximum of 140 mg. at 1.5 hours and returning to 112 mg. at the end of two hours. The glucose curve obtained immediately after stopping the insulin showed a maximum of 192 mg. at the end of one hour, but had fallen to 82 mg. at the end of two hours; the urine was negative for sugar. Although readjustment to normal was prompt in this case, there was evidence of a transitory diminution of tolerance.

E. G., female, aged forty-four years, was admitted to the hospital June 7, 1932. Her complaints were weakness, loss of weight, and a poor appetite. A diagnosis of infectious arthritis and psychoneurosis was made. Insulinization was started on June 10 with 5 units before meals and was subsequently progressively increased to 18 units t. i. d. by June 25. Insulin was discontinued on June 30 with the last injection at noon. The blood sugar at

2 P.M. was 120 mg., but at 7 P.M. was 295 mg. with a glycosuria of 0.14 per cent. A glucose tolerance test the following morning gave a mild diabetic type of curve showing a maximum of 241 mg. at two hours and a glycosuria of 0.54 per cent at one hour and 0.22 per cent at two hours. Although the curve obtained before insulin was started showed a somewhat diminished tolerance with a maximum of 226 mg. at one hour, and 176 mg. at the

TABLE VI

H. J. HOSPITAL NO. G4997

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
6/ 4/32	87*, 1	0- 5- 5	104¼	*Fasting; tolerance
6/ 5	79	5- 5- 5		
6/ 6	131	5- 5- 5		
6/ 7	105	5- 7- 7	106¾	
6/ 8	86	7- 7- 7		
6/ 9	109	7- 7- 7		
6/10	91	7- 7- 7		
6/11	124	7- 8- 8	107⅓	
6/12	118	8- 9- 9		
6/13	105	9- 9- 9		
6/14	104	9-10-10		
6/15	85	10-10-10	109½	
6/16	94	10-10-10		
6/17	108	10-10-10	109½	
6/18	87*, 2	0-10-10	110¼	*Fasting; tolerance
6/19	77	10-10-12	109½	
6/20	109	12-15-15	110	
6/21	85	15-15-15	110	
6/22	159	15-18-18	110	
6/23	58	18-18-18	109½	
6/24	92	18-19-19	110½	
6/25	152	19-20-20	111½	
6/26	33	20-20-20		
6/27	94	20-20-20		
6/28	124	20-20-20	110¾	
6/29	165	20-20-20		
6/30	114	20-20-20		
7/ 1	138	20-21-21	112¾	
7/ 2	157	21-22-22		
7/ 3	155	22-23-23	116½	
7/ 4	110	23-23-23		
7/ 5	73	23-23-23	114½	
7/ 6	107	23-23-23		
7/ 7	159	23-24-24		
7/ 8	155	24-25-25		
7/ 9	90	25-25-25	113¾	
7/10	82	25-25-25		
7/11	87*, 3 140 (1 P.M.) 147 (2 P.M.)			*Fasting; tolerance
7/12	122		113	
7/13	155			
7/15	98			
7/16	114*, 4		113½	*Fasting; tolerance
7/18	104			
7/19	91*, 5			*Fasting; tolerance

*Glucose tolerance: 50 gm. glucose by mouth

Hours	0	0.5	1	1.5	2	2.5	Urine
1B. S. mg. per cent	87	159	126	124	95		Negative
2B. S. mg. per cent	87	182	231	179	100		Negative
3B. S. mg. per cent	87	208	231	253	204	176	1 hr. 0.8% 2 hr. 1.1%
4B. S. mg. per cent	114	204	208	189	99		Negative
5B. S. mg. per cent	91	186	173	147	109		Negative

Blood sugars ordinarily taken at 9 A.M., two hours after breakfast.

end of two hours with no glycosuria, the curve obtained after insulinization showed considerably greater diminution of tolerance. By the second day after insulin was discontinued, the two-hour postabsorptive blood sugar level had returned to about its previous level. The patient's weight had increased from 108 pounds on admission to 112 pounds on June 28, and further increased after insulin was discontinued to 114 pounds by July 7. The patient's appetite was only slightly increased.

H. J., female, aged thirty-four years, was admitted to the hospital June 2, 1932. A diagnosis of pleurisy with effusion was made. She had lost weight and her appetite was poor. Insulin injections were started on June 4 with 5 units before meals, and the insulin was increased a few units at a time at intervals of several days to 25 units t. i. d. Blood sugar values were usually normal, but frequently higher. Insulin was discontinued on July 10 with the last injection at the evening meal. A glucose tolerance before the insulin injections were started was normal with a maximum of 159 mg. at 0.5 hour and 95 mg. at two hours. A second test was done on June 18 when the patient was receiving 10 units of insulin t. i. d.; the blood sugar reached a maximum of 231 mg. at one hour and was 100 mg. at the end of two hours. On July 11 when insulin was discontinued after the patient was receiving 25 units of insulin before each meal, the glucose tolerance curve was of the mild diabetic type reaching a maximum of 253 mg. at 1.5 hours, and was 204 mg. at two hours, and 176 mg. after 2.5 hours with a glycosuria of 0.8 per cent at one hour and 1.1 per cent at two hours. Another glucose tolerance curve five days after insulin had been discontinued showed a maximum of 208 mg. after one hour, but dropped to 99 mg. after two hours. The last tolerance done after eight days while showing a further improvement had not returned to the initial level; the maximum blood sugar was 173 mg. at one hour, and 109 mg. at the end of two hours. The patient's weight had increased from 104 pounds on admission to 113 pounds at the time insulin was discontinued; there was no subsequent gain in weight observed. Her appetite showed considerable improvement.

DISCUSSION

The observations that insulin may be given in progressively increasing amounts without causing hypoglycemia, and that a temporary hyperglycemia and glycosuria associated with a diminution of glucose tolerance is observed upon the sudden discontinuation of the injections suggest a certain endogenous readjustment of the organism to maintain a normal blood sugar level. The most obvious explanation is that there is a compensatory inhibition of the normal islet secretion during the course of insulinization, and a slow readjustment following discontinuation of the insulin until normal activity is attained. Such an interpretation is in accord with the evidence from this laboratory of a latent functional capability of the island cells in diabetes mellitus.[8, 9] Similar observations indicating the suppression of the normal activity of a gland through the exogenous administration of its secretion have been reported with respect to the thyroid, parathyroid, and ovaries. However, a study of the respiratory quotients of insulinized nondiabetic patients has suggested another possible explanation. During and following periods of insulinization we have observed low respiratory quotients indicating an increased combustion of fat. Since Sweeney[10] has shown that high fat diets tend to reduce the glucose tolerance, this factor may possibly be operative in the experiments reported here.

The apparent increases in appetite and body weight have been of the greatest interest clinically. Most observers have reported substantial increases during as well as following a series of daily insulin injections. It is to be noted, however, that in many instances the total caloric intake was increased to as high

as 4,000 calories, so that it is to be questioned whether or not the increased caloric intake in itself would not have produced the same results. An increased gastric motility associated with an increased secretion of gastric juice following insulin injections[11, 12, 13, 14] has been reported by a number of investigators; this may in part explain the increased appetite frequently reported, especially in cachectic states. It would seem advisable that for purposes of stimulating the appetite, the insulin should be given an hour or more before meals. Whether exogenous insulin plays a definite rôle metabolically in producing an increase in body weight remains an open question.

CONCLUSIONS

Insulin may be given in progressively increasing amounts to nondiabetic subjects without producing hypoglycemia.

There is a progressive diminution of glucose tolerance accompanying the increase in the amount of insulin given.

Upon suddenly discontinuing the course of insulinization, there is a resulting transitory hyperglycemia and glycosuria lasting one to three days or even longer.

The data indicate that the insulin given may cause a compensatory inhibition of the normal islet secretion.

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STUDIES ON INDOLURIA*

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THE present investigation was begun in an attempt to determine whether there is any evidence of impairment in the processes of detoxification in the body in chronic arthritis. At the beginning of the investigation, we made quantitative estimations of the amount of indican excreted by patients with chronic arthritis, routine hospital cases excluding arthritic patients, and normal subjects. The amount of indican excreted by 43 normal subjects, as determined by the Sharlit[1] method, averaged 78 mg. per day. Usually determinations were made on twenty-four-hour specimens for three consecutive days. There was considerable variation in the amount found in different persons, the indican ranging from 30 to 165 mg. per day. The average for 157 hospital cases with various pathologic conditions other than chronic arthritis was 67 mg. per day, the amounts ranging from 5 to 260 mg. In the cases of arthritis there was a tendency for a low indican output, the average for 96 cases of arthritis being 44 mg. per day, the amounts ranging from 7 to 358 mg. Although the average indican output seems to be lower in chronic arthritis than in other pathologic conditions, no definite relationship was found to exist between it and the clinical condition of the patient. Hypertrophic cases were more consistently low than rheumatoid cases. The high figures were all found in the latter condition.

Owing to the inconsistency of our results with indican determinations, we turned our attention to the examination of urine for indole, believing that if there were impairment in the processes of liver detoxification, indole might appear as such in the urine. As the results show, indole or a precursor of indole which is converted into indole in an acid solution was found in the urine of most cases of chronic arthritis.

The presence of indole in the urine distillate of normal individuals was reported by Jaffé.[2] However, the amount was very small since one or two liters of urine were necessary for each determination. Similarly, we have found that when urine is distilled directly to a very low volume, as done by Jaffé, indole sometimes appears in the distillates from normal urines. It has also been our experience that the addition of strong mineral acids to the urine before distillation often leads to considerable indole formation. Although small amounts of indole, or of some substance which liberates indole on steam distillation from a solution made acid with an organic acid, are occasionally found in normal urine, they are not present in significant amounts. This is evident from the fact that out of over one hundred determinations on normal individuals, we found urinary indole in only one case and then on only one occasion.

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Becher,[3] upon examination of the urine, found free indole in cases of severe liver insufficiency. Cantelli[4] found indole in the urine of patients with destructive pulmonary lesions, mainly tuberculosis. He attributes indoluria in such cases to putrefaction in the body. Schour and Rosengarten[5] also found that many cases of pulmonary tuberculosis gave a positive result to the test for indole in the urine. In their cases, the amount of indole present was somewhat proportional to the activity of the infection. Those patients with a large amount of indole in the urine usually had an unfavorable later clinical course, while those patients showing a negative or slightly positive test for indole progressed favorably.

Vaughan[6] tried to determine if the power of the liver to detoxify indole could be used as a liver function test. He based his work on the theory that if the liver were unable to detoxify indole because of intrinsic disease, free indole would appear in the urine. Therefore, he tested the urines of patients with various pathologic lesions of the liver, such as acute arsenical hepatitis, acute catarrhal jaundice, subacute yellow atrophy, obstructive jaundice, and jaundice of undetermined origin. He also tested urines from patients with marked intestinal stasis and putrefaction. In no case was he able to detect indole by the method employed, which was a modification of the test with Ehrlich's reagent. Bilirubin was precipitated from the urine with barium chloride, the solution filtered, and a petroleum ether extract of the filtrate treated with Ehrlich's reagent. The difference between Vaughan's results and ours (given below) may be attributed to the difference in the methods employed.

Since the presence of indole or of an indole precursor in the urine in cases of arthritis can hardly be considered as arising from a focus of putrefaction in the body or from a demonstrable liver insufficiency, it was decided to investigate the possible occurrence of indole in various other pathologic conditions in an attempt to explain its presence in arthritis. As our results show, indole was found to be present in most cases of arthritis, toxemia of pregnancy, pellagra, rheumatic fever, tuberculosis, and in many cases of diabetes mellitus.

Experimental.—Method of Analysis: One hundred cubic centimeters of fresh urine, usually the morning specimen, were placed in a 600 c.c. distilling flask, 20 drops of bromphenol blue added, and a 40 per cent aqueous solution of tartaric acid run in until there was an excess of 5 c.c. above the volume necessary to give a color change of the indicator. This usually required approximately 6 c.c. of the tartaric acid solution. The solution was steam distilled until 200 c.c. of the distillate was collected. A small flame was kept under the distilling flask during the distillation to maintain a relatively constant volume of liquid in the flask. The distillate was transferred to another distilling flask, the solution made alkaline to phenolphthalein, and redistilled over a free flame until 100 c.c. had distilled over. To 50 c.c. of this distillate, 1 c.c. of 2 per cent KOH was added, followed by 1 c.c. of approximately 2 per cent sodium B-naphthoquinone-4-sulphonate, and the solution mixed. After standing for ten to fifteen minutes, the solution was extracted with 3 c.c. of chloroform. The presence of indole is shown by a red color in the chloroform layer. The supernatant layer was syphoned off and alcohol containing 1 per cent KOH added to the

chloroform extract up to the 12.5 c.c. mark. The solution then turned a greenish blue color. This was compared colorimetrically with a standard indole solution treated in the same way as the final distillate. A stock solution of indole was made by dissolving 20 mg. of indole C.P. in 100 c.c. of water. This solution will keep for a week or more in the ice box. The dilute standard was prepared from this solution by diluting 5 c.c. to 100 c.c. in a volumetric flask. For the determination 5 c.c. of the dilute standard was placed in a tube, further diluted to 50 c.c., and the color reagents added. The recovery of indole is not totally quantitative owing to incomplete distillation of indole, but the results obtained by the above method should be comparable. Furthermore, we feel that the important factor is the presence of appreciable quantities, and not the absolute daily output, of indole in the urine.

The results obtained from the various urines studied are given in Tables I and II. Values given as traces are less than 0.05 mg. per liter of urine. Very few of those cases giving a positive result showed as low as 0.3 mg. per liter. The majority of them were between 0.5 and 1.5 mg. per liter of urine. It is likely that traces of indole are not of clinical significance.

In Table I is shown the occurrence of indoluria in a number of different diseases. In Table II are shown the urinary indole output in typical cases of several diseases and the variations that accompanied clinical improvement.

TABLE I

URINARY INDOLE IN VARIOUS CONDITIONS

NO. OF CASES	NEG.	TRACE*	POSITIVE	DIAGNOSIS
50	49	1	0	Normals
4	4	0	0	Bronchopneumonia
8	4	2	2	Lobar pneumonia
1	0	0	1	Abscess of lung
3	2	0	1	Peptic ulcer
2	0	0	2	Alcoholic intoxication
2	0	0	2	Acute yellow atrophy of the liver
13	2	0	11	Congestive heart failure
8	0	0	8	Pellagra
2	0	0	2	Cirrhosis of the liver
1	0	0	1	Exfoliating dermatitis
20	1	1	18	Pulmonary tuberculosis
1	0	0	1	Tuberculous peritonitis
12	4	8	0	Upper respiratory infection
8	0	0	8	Diabetes with gangrene
11	5	1	5	Diabetes without gangrene
14	2	0	12	Toxemia of pregnancy
2	0	0	2	Rheumatic fever
5	3	0	2	Gonococcal arthritis
3	0	0	3	Infectious arthritis
24	0	2	22	Rheumatoid arthritis
13	2	1	10	Hypertrophic arthritis
2	0	0	2	Mixed arthritis
34	32	2	0	Various pathologic conditions, such as acute lymphatic leucemia, psychoneurosis, syphilis, pleurisy, typhoid fever, carcinoma of the stomach, arsenical dermatitis, common cold, malnutrition, and Banti's disease.

*A urine was considered to contain only a trace of indole if it showed less than 0.05 mg. of indole per liter of urine.

TABLE II

VARIATIONS IN URINARY INDOLE DURING TREATMENT

PATIENT	DIAGNOSIS	INDOLE	DATE	CONDITION
Mrs. Hu.	Acute lobar pneumonia	1.8	4/16/34	Serious
		4.8	4/19/34	Unimproved
		1.8	4/21/34	Better, temperature down
		1.6	4/25/34	Temperature normal
		0.6	5/ 1/34	In good condition, discharged
Mr. Co.	Lobar pneumonia	0.6	4/18/34	Temperature 106°, serious
		Neg.	4/19/34	Temperature normal, much improved
		Neg.	4/20/34	Continued to improve
Mrs. Bo.	Exfoliating dermatitis	3.2	6/ 5/34	Rash with swelling over entire body
		2.8	6/ 7/34	Slightly better
		0.9	6/16/34	Much better, skin clearing rapidly
		0.5	6/22/34	Continued improvement
		Neg.	7/ 5/34	Skin lesions absent
Mr. El.	Hypertrophic arthritis	0.7	6/20/34	Suffering considerably
		0.9	6/21/34	Unchanged
		0.5	6/22/34	Unchanged
		--	6/23/34	"Arthritis diet"
		Neg.	6/28/34	Improvement remarkable
		0.3	7/11/34	Reappearance of pains following laxity in dieting
		Trace	7/18/34	Much better
		Neg.	7/31/34	Some pain present following recent exposure to cold and damp
		0.5	8/ 7/34	Pains returned following temporary discontinuance of diet
Mr. Ne.	Rheumatoid arthritis	1.0	6/23/34	Pain very severe
		0.7	6/25/34	Unchanged
		1.0	6/26/34	Unchanged
			6/27/34	Pains more severe, placed on "arthritis diet"
		0.8	6/28/34	Feeling better
		0.7	6/30/34	Somewhat improved
		0.5	7/ 5/34	Some improvement
		0.1	7/ 6/34	No pain
		1.4	7/ 8/34	Reappearance of pain, cause unknown
		0.6	7/18/34	Pain only slight
		0.6	7/21/34	Pain slight
		Trace	7/27/34	In good condition
		Neg.	7/29/34	Entirely free from pain
		Neg.	8/24/34	Continued good
			10/ 1/34	Diet discontinued
		1.2	10/15/34	Pains returned, rather severe
		1.1	10/16/34	Unchanged
		0.8	10/17/34	Unchanged, returned to diet
		0.3	11/ 6/34	Much better, but some pain present
		Neg.	12/ 6/34	In good condition, no pain
Mrs. Ar.	Rheumatoid arthritis	0.7	6/ 7/34	Considerable pain
		0.5	6/ 8/34	Unchanged
			6/ 9/34	"Arthritis diet"
		Neg.	6/23/34	Free from symptoms
Mrs. Gr.	Hypertrophic arthritis	0.7	6/20/34	Suffering considerably
		0.9	6/21/34	Unchanged
		0.5	6/22/34	Unchanged
			6/23/34	Placed on "arthritis diet"
		Neg.	6/28/34	Improvement remarkable
		0.3	7/11/34	Some reappearance of pain, following laxity of diet
		Trace	7/18/34	
		Neg.	7/31/34	Some pain present following recent exposure to cold and damp
		0.5	8/ 7/34	Pain following temporary discontinuance of diet

TABLE II—CONT'D

PATIENT	DIAGNOSIS	INDOLE	DATE	CONDITION
Miss Si.	Pellagra	--	3/ 3/34	Treatment begun, advanced case
		2.0	3/ 6/34	Very sick
		Trace	3/10/34	Very rapid improvement, skin lesions clearing
		0.1	3/17/34	Good
		0.2	3/18/34	Good
		Neg.	3/31/34	Symptom-free, excellent condition
Mrs. De.	Spondylitis	0.5	5/ 2/34	Moderately severe case
		0.3	5/ 5/34	Some improvement
		Trace	5/ 8/34	Continued improvement
Mr. Whi.	Congestive heart failure	2.4	7/ 8/34	In serious condition, much decompensation Given digitalis, with good response
		Trace	7/11/34	Very much better
		0.5	7/16/34	Continued good
		Neg.	7/18/34	In good condition
Mrs. St.	Toxemia of pregnancy	1.0	7/10/34	Serious condition
			7/11/34	Spontaneous delivery of normal child
		0.5	7/19/34	Improving rapidly
		0.4	7/21/34	Improving rapidly
		Trace	7/23/34	Continued to improve
		0.2	7/27/34	Good
		Neg.	7/29/34	Discharged in good condition
I. T.	Toxemia of pregnancy	Trace	7/13/34	Severe toxemia of pregnancy in fourth month
		2.8	7/15/34	Condition serious
		2.4	7/17/34	Unchanged
		0.5	7/18/34	Better
		0.7	7/18/34	Regaining strength
		0.9	7/19/34	Stronger
		--	7/30/34	Sent home to return for delivery
		0.8	11/ 7/34	Returned in labor, very sick
			11/ 8/34	Delivered
		Neg.	11/10/34	Very much better
		Neg.	11/11/34	Rapidly regaining strength
F. S.	Toxemia of pregnancy	--	11/25/34	Patient very ill
		--	11/26/34	Spontaneous delivery of normal child
		0.8	11/27/34	Uneventful and rapid recovery
		Neg.	11/29/34	Discharged on December 6
		Trace	11/30/34	
		Neg.	12/ 1/34	
		Neg.	12/ 2/34	
Mrs. H.	Toxemia of pregnancy	--	7/29/34	Spontaneous delivery of normal child
		1.1	7/30/34	Slow but gradual recovery after delivery. Discharged in good condition on August 9.
		3.0	8/ 1/34	
		0.4	8/ 2/34	
		1.2	8/ 4/34	
		3.5	8/ 5/34	
		2.0	8/ 6/34	
		1.1	8/ 8/34	

Discussion.—Indole may originate in the body from two sources: from bacterial decomposition of tryptophane in the intestinal tract and from putrefactive foci. Indole is normally detoxified in the liver by conversion into indoxyl and conjugation with monopotassium sulphate to form indican. Laroche and Desbordes[7] injected indole into the portal vein of dogs and determined at intervals the amounts of indole and of indoxyl present in the hepatic vein. Blood taken from the hepatic vein immediately after the injection of indole

gave a positive test for indole but not for indoxyl. Ten minutes later indoxyl began to replace indole, large quantities of indoxyl being found in the hepatic vein even up to forty minutes after the injection of indole. Similar experiments were performed on excised liver, kidney, and lung tissues after perfusing them with Ringer's solution. The liver only was found capable of oxidizing the indole to indoxyl.

Sulphur is a major factor in the detoxification of indole. The conjugation of indoxyl with the sulphate radical forming the ethereal sulphate, indican, most likely takes place in the liver. Hele,[8] working with dogs, found that the ethereal sulphate was increased in the urine after administration of indole. He believes that the sulphur used by the body for detoxification is probably derived from the sulphate ions in the cell fluids, and that, as this is depleted, the sulphate ions are replaced by those formed from the oxidation products of metabolism. In the dog, this resulting sulphur deficiency can be replenished by the oral administration of sulphate, but in the pig, orally administered sulphates have no detoxifying action.[9, 10]

It is still unsettled whether sulphates as such are of value in the detoxification processes in man. Voegtlin, Johnson and Dyer[11] have shown that cystine, cysteine, and glutathione all protect the albino rat from minimal lethal doses of cyanide. Agnoli[12] found that colloidal sulphur exerted a definite antitoxic effect against minimal lethal doses of diphtheria and botulinus toxins. Waelsch[13] found that the narcosis produced by avertin could be avoided by the administration of glutathione or several other sulphur compounds. These investigations tend to show that certain sulphur compounds are important factors in the protection of the body against toxic compounds and in the elimination of these compounds from the body.

On examination of Tables I and II we find that indoluria may be present in patients suffering from the following pathologic conditions: lobar pneumonia, severe alcoholic intoxication, liver atrophy, congestive heart failure, pulmonary tuberculosis, diabetes mellitus with and without gangrene, pellagra, toxemia of pregnancy, and chronic arthritis. To explain the presence of indoluria in this list of diseases is not a simple matter. Several of these conditions are of questionable origin, and the presence of urinary indole in them may be the result of similar etiologic factors, as yet unknown. Theoretically, indoluria might result from at least three conditions, first, absorption of indole from the intestinal tract in such large amounts that the normal liver is unable to cope with it all; second, such impairment of the liver function that it is incapable of detoxifying even that amount which is absorbed normally from the intestinal tract; and third, indole arising from some area of the body other than the intestinal tract, and being excreted by the kidney without having passed through the liver.

Indole in the urine of tuberculous patients, as reported by Cantelli[4] and Schour and Rosengarten[5] and confirmed by us, may be due to a definite focus of putrefaction in the lung, as suggested by Schour and Rosengarten. Indole found in the urine of severe cases of lobar pneumonia might have a similar

origin, although the well-known toxic degeneration of liver cells in this condition is a more probable explanation. Another possible explanation for the indoluria of tuberculosis is that, due to the lowering of general resistance, the liver is unable to detoxify the indole absorbed from the intestinal tract so that some of it passes to the kidneys and appears in the urine. In diabetes with gangrene, the indoluria may be attributed to the putrefying and gangrenous area.

The explanation of indoluria in cases of congestive heart failure, severe liver damage, and toxemia of pregnancy is clear. Since the liver is the seat of indole detoxification, any impairment of its function by congestion or damage to the organ itself would be expected to allow indole to pass through unchanged.

The presence of indole in the urine in cases of arthritis, diabetes without gangrene, and pellagra is not so easily explained. It is not clear whether we are dealing with a greatly increased formation of indole in the intestinal tract or with a decreased power of the liver to detoxify the indole normally formed and absorbed or with a combination of both. The possibility of indole formation in some focus outside of the intestinal tract can hardly be considered a factor in these diseases. We are as yet in no position to prove or to disprove either of these possibilities.

It is of interest to note that when patients with chronic arthritis, pellagra, congestive heart failure, or toxemia of pregnancy have improved, there has been a reduction in the amount of indole excreted parallel, as a rule, with the clinical improvement. In several cases of toxemia of pregnancy, indoluria continued for several days after delivery even after the clinical symptoms of toxemia had apparently disappeared. We have, at present, no plausible explanation for this.

The mechanism by which the indoluria is eliminated in pellagra and arthritis is still unknown, but it is of interest to note that the ordinary pellagra diet, as well as the diet used in the treatment of the arthritic patients reported here, is high in both sulphur and vitamin B_2. We are tempted to believe that, especially in arthritis, the high sulphur content of the diet was a factor in bringing about improvement. A detailed clinical report of our results on the treatment of arthritis with this diet will be published later.

SUMMARY

Urine from normal subjects and from patients with various pathologic conditions was examined for indole. Indole was found in significant amounts in many cases of chronic arthritis, pellagra, diabetes mellitus, tuberculosis, toxemia of pregnancy, congestive heart failure, and lobar pneumonia, but not in the urine of normal subjects. A short discussion dealing with the mechanism of indoluria is given.

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The clinical diagnoses of the cases here reported were made by the clinical staff of the college hospitals. We take pleasure in acknowledging their cooperation in this matter, as well as for their many helpful suggestions during the investigation.

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A STUDY OF CORYNEBACTERIUM DIPHTHERIAE AND RELATED ORGANISMS IN MARYLAND*

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INTRODUCTION

THERE is considerable disagreement among bacteriologists as to the taxonomy of the species *C. diphtheriae*. Any attempt to classify this species solely on the basis of morphology is obviously impractical. No one can describe or picture the numerous variations that *C. diphtheriae* exhibits when grown on media of only slightly varying composition and in association with other organisms. While the organisms can, in many instances, be readily recognized solely by their morphology and staining characters on certain media, there are instances in the examination of Löffler slant cultures from the throat or the nose where it is impossible for the most skillful observer to be sure whether certain bacteria are *C. diphtheriae*, diphtheroids or neither. There can be no doubt that a certain percentage of cultures examined routinely in bacteriologic laboratories have been reported negative when *C. diphtheriae* has been present or reported positive when only diphtheriods were actually found. The question is how can one distinguish between *C. diphtheriae* and diphtheroids? Failure to diagnose *C. diphtheriae* may be serious, for a physician depending on

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laboratory service may fail to administer antitoxin or may release cases still harboring virulent *C. diphtheriae* to spread infection to others. Healthy carriers of virulent organisms may also not be recognized and continue to spread infection. Examinations in which diphtheroids are mistaken for *C. diphtheriae* may result in unnecessary quarantine with economic loss or loss of school attendance. A satisfactory settlement of this problem has been prevented in large measure because of reliance on such elusive and indefinite grounds as morphology. Nearly every experienced bacteriologist has his own rather definite opinion as to what is or is not *C. diphtheriae*, morphologically. Resort, therefore, to checking one person's observations against another's, while helpful, has too often been indecisive, for opinion is not fact. Testing the toxicity of a culture for guinea pigs has also its limitations in determining if a disputed organism is or is not *C. diphtheriae*, for nonvirulent strains are well recognized.

Fermentation reactions seem to offer a most practical means of differentiating *C. diphtheriae* from morphologically similar organisms.

PURPOSE OF STUDY

One purpose, therefore, of this study was to determine if possible the dependability of fermentation reactions in distinguishing *C. diphtheriae* from similar morphologic forms. In the study of fermentation tests, an attempt was made to determine if such reactions were constant; to try to explain discrepancies that were obtained by some investigators; to find out if fermentation reactions correlated with virulence tests; to distinguish between fermentation reactions of *C. diphtheriae* and diphtheroids; and to find out if sugar fermentations correlated with colonial and other distinctive characters of *C. diphtheriae* types.

Another phase of this study is concerned with the recognition of different varieties of *C. diphtheriae*.

CHARACTER AND SOURCE OF MATERIAL

All cultures used in this study were isolated from Löffler slant cultures inoculated from throat and nose swabs as routinely examined in the Bacteriological Laboratories of the Maryland State Department of Health. Since nose cultures were not routinely made for diagnosis in these laboratories until the past year, the majority of cultures studied have been from the throat. These cultures were secured from clinical cases of diphtheria, convalescents, contacts, and healthy carriers.

SOURCE OF CULTURES STUDIED

TABLE I

	NO. OF STRAINS	THROAT	NOSE	EAR
1. *C. diphtheriae*	111	107	4	0
2. Diphtheroids	50	47	2	1

METHOD OF ISOLATION OF PURE CULTURES

The isolation of pure cultures of *C. diphtheriae* is not always an easy matter. In stained preparations, contaminations may be so few in some instances

that they may escape detection. Pleomorphism is so characteristic of *C. diphtheriae* that the contaminant though present may be taken for one of the individual cells of *C. diphtheriae* in the stained smear. On a few occasions, an apparently typical colony of *C. diphtheriae* has been found to contain a contaminating organism. The contaminants in such cases were staphylococcus, streptococcus or very rarely a diphtheroid. Hence, every culture studied has been carried through at least two subcultures before considering it pure. Insufficient care in purification and isolation has probably been responsible for some of the disagreements that different investigators have obtained in their studies on fermentation reactions.

In the isolation of cultures, infusion broth was inoculated with a loopful of material from Löffler slant containing the mixed culture and incubated at 37° C. for eighteen to twenty-four hours. A potassium tellurite plate was then streaked with a small loopful taken from the surface of the broth in such a manner as to secure *widely separated* colonies. Löffler medium was not used for plating directly from mixed cultures, as it was found subject to liquefaction and spreading by certain contaminating organisms, and too, colonies of *C. diphtheriae* were not so easily distinguished as on the tellurite medium. Several characteristic colonies from the forty-eight- to seventy-two-hour tellurite plates were picked to Löffler slants in order to secure different types if these were present and to avoid, if possible, the chance of selecting avirulent strains. As final proof that cultures were pure, tellurite plates were streaked from each individual Löffler slant culture. Portions of the same colony on a tellurite agar plate were transferred respectively to another Löffler slant to make ready for inoculation into fermentation tubes and to an agar slant for reserve. The agar slant culture was an additional check on purity since contaminants, if present, could be more easily detected in the growth on an agar slant which is thin, than on a Löffler slant which gives a more profuse growth.

FERMENTATION REACTIONS OF C. DIPHTHERIAE

The fermentation reactions of *C. diphtheriae* have been a controversial subject since the publication of Hine's statement in 1913 that he "had yet to find an organism of the diphtheria group giving acid with glucose and dextrin and not with saccharose which proves to be other than *B. diphtheriae*." It was found in this study that filtered sugars gave constant reactions, whereas heated sugars were unreliable in many instances. The following filtered sugars were used for the reactions: dextrose, dextrin, maltose, sucrose, starch, and glycogen. The reactions in Hiss serum water were found so inconstant and hence unreliable, in the first part of this study, that its use was discontinued and extract broth was substituted. After substituting extract broth containing 1 per cent of carbohydrate and indicator, no inconstant reactions resulted.

Transfers from twenty-four-hour Löffler slants were made into tubes containing the individual carbohydrates and incubated at 37° C. Readings were taken on the first four days and again on the seventh and tenth days. In some instances, readings were noted on the fourteenth, twenty-first, and twenty-eighth days. Uninoculated tubes were always incubated along with the tests as con-

trols. With the majority of strains of *C. diphtheriae*, definite though not always complete reactions were observed in twenty-four hours. Maximum reactions were reached within from forty-eight to seventy-two hours. The amount of acid produced by some strains from dextrin was considerably less than that formed from dextrose.

That *C. diphtheriae* ferments dextrose and fails to affect sucrose is recognized by most investigators. However, L. Martin (1898), Graham-Smith (1908), Moshage and Kolmer (1916), Cary (1917), Durand (1921), Fitzgerald and Doyle (1923), claim to have isolated *C. diphtheriae* strains which fermented sucrose. On the other hand, A. Knapp (1904), Zinsser (1907), Rothe (1907), Arkwright (1908), Morse (1912), Neisser and Gins (1913), Hine (1913), Guthrie, Gelien and Moss (1920), Jordan, Smith and Kingsbury (1922), Barrett (1923), Engering (1923), Christianson (1923), OKell and Baxter (1924), have failed to observe sucrose fermentation by *C. diphtheriae*.

In the present study, constant fermentation reactions were produced by all strains of *C. diphtheriae*. Dextrose, dextrin, and maltose were fermented by all the one hundred and eleven strains. Starch and glycogen were fermented by seven strains in addition to the above-mentioned sugars. Sucrose was not fermented by any of the *C. diphtheriae* strains. Dextrin was found to be weakly fermented by some strains. In some instances, it has been found to produce acid in twenty-four hours and then revert to an alkaline reaction. Both virulent and avirulent strains of *C. diphtheriae* fermented dextrin (see Table II). No difference was found in the fermentation reactions of virulent and avirulent strains of *C. diphtheriae*.

CULTURAL CHARACTERISTICS OF VARIOUS TYPES OF C. DIPHTHERIAE AND THE DIPHTHEROIDS

Colony Morphology of Types of C. Diphtheriae and Diphtheroids on Tellurite Agar Plates.—Three types of *C. diphtheriae* colonies were observed on tellurite serum agar plates. These corresponded to the gravis, mitis and intermediate types described by Anderson, Happold, McLeod and Thomson (1931).

Gravis colonies: Colonies corresponding to the gravis type appear after forty-eight to seventy-two hours of incubation at 37° C. as dull, grayish black (nonglistening). They measured from 3 to 3.5 mm. when well isolated. The colonies were irregular in outline and had a tendency toward flatness and spreading. Radial lines were usually noticeable after forty-eight hours incubation and became more pronounced later. These features are responsible for the rough appearance of the gravis type of colony. *Mitis colonies:* Colonies corresponding to the mitis type were about the same size as the gravis but differed by being glistening black, round, and regular in outline and by having convex, smooth surfaces. *Intermediate colonies:* Colonies resembling the intermediate type were very small and dull gray with a raised black dot center at twenty-four hours. After forty-eight hours, the colonies became grayish black, rough and flat with a finely serrated edge. The intermediate colony usually measures about 0.5 mm. in twenty-four hours and from 1.00 to 1.25 mm. in forty-eight hours. The colonies are much smaller (about one-half the diameter)

than the other two types. They resemble those produced by streptococci. No variation was noticed in the colony morphology of any of the three types subcultured frequently over a period of seven months.

Colonies of diphtheroids showed much variation on tellurite serum agar, also. The majority of strains produced a small, dead white colony in twenty-four hours, which in forty-eight hours increased in size, became gray, smooth and moist. Other strains produced thin, transparent colonies of a light grayish brown coloring which in forty-eight hours became somewhat larger and duller in color. A few strains produced small flat, colorless colonies in twenty-four hours which, in forty-eight hours, became dry and brownish black in color.

Colony Morphology of C. Diphtheriae and Diphtheroids on Löffler Medium.—The general morphology of the three types of *C. diphtheriae* was similar on Löffler medium but was less characteristic than on tellurite serum agar as no color changes occurred.

The diphtheroid colonies like *C. diphtheriae* colonies were not so easily differentiated on Löffler medium as on the tellurite medium except when pigmented.

Cellular Morphology of C. Diphtheriae and Diphtheroids on Löffler Medium.—In this study, smears stained with methylene blue were made from cultures of *C. diphtheriae* grown on Löffler slants which had been incubated for eighteen to twenty-four hours. The cellular morphology of a single strain varied with the different batches of Löffler medium. It was found that the morphology varied all the way from "typical" *C. diphtheriae* to that of forms unrecognizable as *C. diphtheriae*. My experience has convinced me that a great deal of error has resulted in laboratories doing routine diphtheria work due to dependence for identification on cellular morphology of cultures grown on Löffler medium, the reasons for which will be pointed out later. The emphasis of textbook writers, teachers, and practical workers alike has been so much on cellular morphology that most laboratory workers have accepted an opinion based on cellular morphology and staining as beyond question. By checking identification of this sort against that based on fermentations, virulence, etc., errors of over 65 per cent have been encountered. There is great need for appreciation of the fact that the routine identification of *C. diphtheriae* is a difficult matter. It calls for painstaking study based not only on cellular morphology but also supplemented by study of cultural reactions. *Gravis type:* the gravis type did not produce the predominant, club-shaped (so-called typical *C. diphtheriae*) forms usually characteristic of the mitis type. This type usually took the methylene blue stain less heavily than the mitis type, and as a result the general impression was that of a lighter blue. Few or no metachromatic granules were produced by the gravis type. *Mitis type:* On the other hand, many metachromatic granules were usually found in the mitis type. As a rule, the mitis strains gave the "typical" *C. diphtheriae* morphology in that there was much pleomorphism, many metachromatic granules, characteristic arrangement, and much variation in length. *Intermediate type:* The morphology of the intermediate type was similar to that of the gravis type.

It is a well-known fact that the morphology of many strains of corynebacteria varies with the age of growth and the medium on which it is grown.

On various batches of Löffler medium prepared under apparently the same conditions, however, variations in morphology of the same cultures have been enormous. In some instances, the change in morphology from batch to batch of medium has been so great as to make the identification of the cultures doubtful if not impossible if fermentations and other reactions had not been made. These variations have been found due to the method of preparation of the medium, the amount of moisture, the pH of the medium, the length of the incubation period, the portion of the slant from which it was taken, influence of other bacteria present and other possible factors. Some strains were found to have quite long forms on one batch of medium, other strains were of medium length, while some were very short. On some batches of Löffler medium, the very short forms would have been indistinguishable from diplococci if there had not been present a few pleomorphic forms. The long, medium, and short forms differed to some extent in their capacity to retain the methylene blue stain. Mitis strains took the stain rather deeply and uniformly with prominent metachromatic granules while other strains (gravis and intermediate) took the stain comparatively lightly and the metachromatic granules were relatively few or absent. Many variations of *C. diphtheriae*, such as dumb-bell, club, etc., may be seen in cultures at times. Usually the most typical morphology (pleomorphism) is obtained on Löffler medium which supports only scanty growth. While typical morphology is desirable for identification it is obvious that a medium supporting only scanty growth of *C. diphtheriae* is not entirely satisfactory for often good growth of other mouth or nose bacteria occurs so that it is almost certain in such instances that *C. diphtheriae* will be overgrown. Some pleomorphism must, therefore, be sacrificed in order to obtain reasonably satisfactory growth. With cultures of diphtheroids one form usually predominates. Rarely is this true of *C. diphtheriae*, for some variations in form and length are seen in any culture. It was not possible to group the strains according to Wesbrooke's classification.

Morphology of C. Diphtheriae on Infusion Agar.—On infusion agar, the morphology of *C. diphtheriae* cultures in general varied somewhat from that on Löffler medium in that the organisms were shorter, heavier, showed fewer metachromatic granules, and often showed a greater tendency toward pleomorphism. The gravis and intermediate strains in contrast to the mitis showed a morphology that was strikingly different in that they were much shorter, produced few or no metachromatic granules, staining was more uniform and a lighter blue. In other words, the gravis and intermediate morphology more closely resembled that of a diphtheroid (*C. xerosis*) than it did the mitis type of *C. diphtheriae* with its metachromatic granules, pleomorphism, and light and dark staining areas.

Morphology of C. Diphtheriae on Potassium Tellurite Agar.—The morphology of *C. diphtheriae* on tellurite agar was similar to that on infusion agar, except there was a more dwarfed appearance in the gravis and intermediate types in that spindle and bottle shapes were predominant.

Growth of C. Diphtheriae in Infusion Broth.—In infusion broth, the gravis type produced a definite pellicle, granular in structure. The supernatant broth was clear with a granular deposit. This contrasted to the mitis type which

showed little or no pellicle, a moderately granular sediment, and a uniform turbidity in twenty-four hours. The intermediate type likewise showed little or no visible pellicle, had a clear supernatant broth, and had a finely granular sediment. After forty-eight hours' incubation, these observations were decidedly pronounced. The presence or absence of a pellicle can best be observed by holding the tubes up to the light and viewing them from below, diagonally.

Growth of C. Diphtheriae and Diphtheroids on Infusion Agar Slants.—Infusion agar was used as another medium on which to study the growth of the different strains of *C. diphtheriae*. A dull (nonglistening) heavy, rough growth was produced by the gravis type in contrast to the glistening, moderate, smooth growth of the mitis form. A sparse, nonglistening, rough growth similar to the growth of a streptococcus was formed by the intermediate type.

Though the growth of diphtheroids is usually indistinguishable from that of *C. diphtheriae* on infusion agar, at times the character of growth of certain diphtheroids has been very helpful in distinguishing them from *C. diphtheriae*. A thick growth whether moist, wrinkled, pigmented, or sticky is very unlikely to be a culture of *C. diphtheriae*.

Hemolysis of C. Diphtheriae Cultures.—Hemolysis of cultures of *C. diphtheriae* was determined by plates made with sheep blood. Very slight hemolysis was manifested by the gravis strains in twenty-four hours as contrasted with the mitis which produced a clear zone. However, on further incubation definite hemolysis was produced by the gravis type. This is in contrast to the findings of Anderson, McLeod, Happold, and Thomson (1931). There was no evidence of hemolysis in the small number of intermediate strains studied. This lack of hemolysis has been suggested by Christison (1933) as a means of differentiation of the intermediate type. Hemolysis was produced by all of the mitis strains tested.

Fermentation Reactions of C. diphtheriae and Diphtheroids.—All fermentation reactions of the gravis, intermediate, and mitis types remained constant. With the gravis strains there was always strong acid production in dextrin, starch, and glycogen in twenty-four hours at 37° C. This appears to be a very constant and stable character and always correlated, in the strains studied, with the rough colony structure. The intermediate strains (few in number) gave the same sugar reactions as the mitis types in that they fermented dextrose, dextrin, and maltose. Sucrose was never fermented by any of the *C. diphtheriae* strains.

There is apparently no correlation between the cellular morphology of diphtheroids and certain fermentation reactions. The differential sugars that are of value are maltose, sucrose, and to a slight extent dextrose, which merely separates the nonfermenting group. Of the 50 diphtheroid strains, 8 fermented dextrose, maltose, and sucrose; 3 fermented dextrose and maltose; 3 fermented dextrose and sucrose; 13 fermented dextrose only; and 23 were nonfermenters (see Table III).

Dextrin was not fermented by any strains of diphtheroids so that the value of dextrin in differentiating the diphtheroids and *C. diphtheriae* has un-

usual significance. As has been stated above, diphtheroids in pure culture can be differentiated from *C. diphtheriae* on morphologic grounds in the majority of instances as there are sufficient pleomorphism, characteristic arrangement and staining properties in *C. diphtheriae* to distinguish it from the uniformity, parallelism, and comparative evenness of staining of diphtheroids. On the other hand, in mixed cultures *C. diphtheriae* and diphtheroids seem to be more subject to variations in morphology than is seen in pure culture, and the personal element enters largely into the diagnosis of such cultures. It is in such cases as these where doubt exists as to the identity of certain forms that isolation of pure culture should be made and the fermentation reactions determined.

Virulence of C. Diphtheriae and Diphtheroids.—In reviewing the literature on the subject of virulence, it is apparent that there are many difficulties to be encountered before a definite opinion can be made as to the pathogenicity of a member of the corynebacterium group. Some investigators state that *C. diphtheriae* can be recognized by staining characteristics and morphology; others use sugar fermentations as a means of differentiation; but all agree that the final criterion of virulence is animal inoculation. Some investigators have considered all organisms resembling *C. diphtheriae* isolated from cases of diphtheria as of etiologic significance. It is possible that a culture may contain avirulent as well as virulent organisms, or it may contain only the avirulent organisms. In order to avoid the danger of selecting an avirulent strain of *C. diphtheriae*, several colonies were picked from the plate first inoculated.

Virulence was tested intradermally by injecting from 0.10 c.c. to 0.15 c.c. of a suspension of a twenty-four-hour growth from a Löffler slant in 5 c.c. of normal saline solution. Guinea pigs weighing between 300 gm. and 450 gm. were used for the tests. The control guinea pig received from 250 to 500 units of diphtheria antitoxin twenty-four hours previous to the time control and test guinea pigs were inoculated with the cultures. Four to six strains of the mitis type of *C. diphtheriae* were usually used on a guinea pig. The gravis and intermediate strains were tested singly. The range of virulence seemed to be greater in the mitis strains while the gravis were more uniform. Some mitis strains gave just as marked reactions on guinea pigs as the gravis and intermediate types, but they did not appear so toxic. Typical reactions with the mitis strains occurred when reddish halos with edema appeared around the point of injection and gradually faded into the natural color of the skin for about half an inch from the centers of injection. These color reactions appeared in twenty-four hours and reached their height in forty-eight to seventy-two hours, while controls showed no reaction. Autopsy findings for gravis strains revealed primarily congested and very hemorrhagic suprarenals with considerable serous fluid in the pleural and pericardial cavities. Guinea pigs inoculated with the intermediate strains showed the same reactions as for the gravis strains.

With regard to the virulence of the three types for guinea pigs, the results are in accord with those of Parish, Whatley and O'Brien (1932), in that some mitis strains are as pathogenic as the gravis strains (i.e., they kill just as quickly). However, on the whole it seems that the gravis strains appear

much more toxigenic or invasive, since all test guinea pigs died in forty-eight to seventy-two hours. The intermediate strains produced somewhat smaller amounts of edema, erythema, and necrosis on guinea pigs than the gravis strains, and they were not as toxic, as the guinea pigs did not die within seventy-two hours. In no case did any of the guinea pigs which were used as controls (i.e., injected with cultures and Park 8 antitoxin) die. Hence, it would appear that the antitoxin protects to the same extent for all types of *C. diphtheriae*. Parish, Whatley, O'Brien (1932) and Povitzky, Eisner and Jackson (1933) found the same results in testing the effectiveness of standard diphtheria antitoxin.

TABLE II

CULTURES OF C. DIPHTHERIAE

NO.	AGAR SLANT	DEXTROSE	DEXTRIN	MALTOSE	SUCROSE	STARCH	GLYCOGEN	VIRULENCE
102 (Mitis)	Smooth, moderate white growth	A	A	A	–	–	–	93+ 9–
7 (Gravis)	Rough, heavy white growth	A	A	A	–	A	A	+
2 (Intermediate)	Rough, fine, white growth	A	A	A	–	–	–	+

TABLE III

CULTURES OF DIPHTHEROIDS

NO.	DEXTROSE	DEXTRIN	MALTOSE	SUCROSE	STARCH	GLYCOGEN	VIRULENCE
23	–	–	–	–	–	–	–
13	+	–	–	–	–	–	–
3	+	–	+	–	–	–	–
3	+	–	–	+	–	–	–
8	+	–	+	+	–	–	–

That the morphology of *C. diphtheriae* has no known relation to virulence has been often demonstrated, and as has been stated above, cultural reactions also do not distinguish between virulent and avirulent *C. diphtheriae*. Hence it seems that virulence, so far, can only be determined by animal inoculation. In testing virulence of *C. diphtheriae* cultures, the intracutaneous method was found to be as reliable as the subcutaneous method. In the intradermal tests made, there were nine avirulent mitis strains. One of these was from a diagnostic case and the others were from healthy carriers.

Intracutaneous tests were made on all diphtheroid cultures. These tests were carried out in the same manner as for *C. diphtheriae* cultures. There was no indication of virulence in any of the cultures tested in this way.

Correlation of C. Diphtheriae Types with Clinical Symptoms.—Even though a comparatively small number of strains of gravis and intermediate types have been found in this study, there appears to be some correlation of morphologic colony types and clinical symptoms. All gravis and intermediate strains were isolated from fatal, very severe cases, or family contacts. These cultures showed very definite and characteristic rough, radial colonial morphology in

mixed culture platings as well as in pure culture. In no instance was there a mixture of types on the plates from which the pure cultures were fished.

SUMMARY

1. In this study of *C. diphtheriae* and related organisms, forms similar to those described by Anderson, Happold, McLeod and Thomson (1931) have been found in Maryland. While only a few gravis (7) and intermediate (3) strains were isolated, the cultural reactions were as follows:

a. *C. diphtheriae gravis* grows with a granular deposit and pellicle in broth; ferments dextrose, dextrin, maltose, starch, and glycogen; forms large lusterless and flattened colony with radial lines; produces hemolysis.

b. *C. diphtheriae mitis* produces uniform turbidity in broth; ferments dextrose, dextrin, and maltose, and it fails to ferment starch and glycogen; forms entire, smooth, convex, glistening colony; produces hemolysis.

c. *C. diphtheriae intermediate* produces a fine granular sediment but little or no visible pellicle in broth; the supernatant is clear; ferments dextrose, dextrin, and maltose but does not ferment starch and glycogen; forms a small, rough, nonglistening, flat colony; produces no hemolysis.

2. The (3) forms of *C. diphtheriae* have remained constant even though transferred at intervals during a period of seven months.

3. Potassium tellurite plate medium was found of great value in differentiation of types. This medium was also found suitable for the growth of delicate as well as hardy forms of *C. diphtheriae.*

4. There was no evidence of mixtures of types of *C. diphtheriae* when original (mixed) cultures were plated out on the tellurite medium.

5. Fermentation reactions were constant for all *C. diphtheriae* strains. Dextrin was found to be weakly fermented by some mitis strains.

6. Fermentation reactions did not offer any differentiation between virulent and avirulent strains of *C. diphtheriae.*

7. Morphology does not distinguish between virulent and avirulent strains of *C. diphtheriae.*

8. It is difficult, if not impossible, to distinguish between *C. diphtheriae* and some diphtheroids in mixed cultures on morphology alone. The distinction is much less difficult in pure cultures.

9. Dextrin was found of value as a differential sugar for *C. diphtheriae* and diphtheroids.

10. No constant relationship was found to exist between morphology, fermentation reactions, and source of diphtheroids.

11. None of the diphtheroids tested showed any virulence.

12. Diphtheroids can frequently be distinguished from *C. diphtheriae* in mixed cultures on Löffler slants. Where doubt exists, the identity can nearly always be established after growth on a tellurite serum agar plate by colonial and cellular morphology. Rarely is it necessary to inoculate sugar broth tubes or make virulence tests to identify cultures correctly.

NOTE: Further study of *C. diphtheriae* strains is being made, which will be reported later.

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MINOR HEMAGGLUTININS*

Study of a Single Human Blood Containing Autoagglutinin, Heteroagglutinins, and Hemolysins, and a Rouleau-Forming Substance

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HUMAN blood agglutinins (other than the familiar isoagglutinins) have been the subject of a number of interesting studies.[1-11] These include the heteroagglutinins (and hemolysins); subgroups of the isoagglutinins; the normal cold agglutinin of Landsteiner; the pathologic cold agglutinin, or panagglutinin, or autoagglutinin; and the rouleau-forming substance (or property). The distinguishing characteristics of the several substances are not entirely agreed upon. In particular a difference of opinion exists as to whether the pathologic cold agglutinin and the rouleau-forming substance are the same. Landsteiner's normal cold agglutinins and the pathologic cold agglutinins do not clearly differ except that the latter are often of high titer, and the former have some of the characteristics of the subgroup isoagglutinins. The presence of four of these substances in a single human blood offers an unusual opportunity to compare their characteristics. The subgroup isoagglutinins, however, are not here considered.

The subject was a white male of twenty years, otherwise healthy, who suffered a typical attack of infectious mononucleosis of seventeen days' duration. Recovery was uneventful. He had no history of allergy. His blood was Type II. Three samples of blood were obtained during the first month of convalescence for the present study. As the reactions were practically the same in all three, they are reported as one study. The heterophile sheep antibody titration according to the method of Paul and Bunnell[12] at the height of the illness was 1:32 for complete hemolysis and 1:256 for the agglutination.

Technic.—The subject's venous blood was allowed to clot at 37° C., and the serum removed before more than slight chilling could occur. This precaution was necessary to preserve the autoagglutinin in full strength in the serum, as this agglutinin adheres to the cells when chilled. The horse and sheep cells were from defibrinated blood and were several days old; the other cells were received at once into a 1.5 per cent solution of sodium citrate in normal saline. All cells were washed twice in warm (37° C.) saline and were used in a suspension of 1 per cent. Sterile precautions were used in preparing and keeping the materials, and all tests were done on the day of collection of the blood, or the day following.

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Titrations were done by the usual method of progressively doubling the dilutions of the serum and adding an equal amount of the cell suspension (0.5 c.c.). Agglutination was determined by placing a drop of the well-mixed suspensions under the microscope. The titers read one to two tubes higher than when read with the naked eye. The slide method of observing agglutination, the reversal of agglutination and the appearance of rouleaus, consisted of making light suspensions of cells in serum (about 1 per cent) on clean microslides ringed with a wax pencil. Slides placed in the 37° C. incubator were within a moist chamber to prevent drying. These and the slides in the refrigerator were taken out for observation one at a time so that the results could not be affected by a change in temperature to that of the room. Observations were made with the microscope, although the agglutinations were easily visible to the naked eye.

Lecithin has been shown by Lattes[13] to prevent rouleau formation. It was used here as a rather heavy suspension of a pure product in normal saline.

The serum and cells of the subject are designated as R serum and R cells. Type IV is that of the Moss classification.

ISOAGGLUTININ

Protocol 1.—Two 5 c.c. portions of R serum, one heated to 56° C. for thirty minutes, and one unheated, were absorbed with 1 c.c. of R cell mush (packed cells) at 5° C. for one hour. The mixtures were shaken several times. They were centrifuged in tubes surrounded by crushed ice and the serum pipetted off quickly. These are designated Solutions A. They failed to agglutinate R cells and Type IV cells after thirty minutes at 5° C., although moderate rouleau formation was noted. Removal of the autoagglutinin was considered complete (see Table I).

TABLE I

TITERS WITH TYPE III CELLS

	37° C. 1 HR.	10° C. 1 HR.	37° C. 1 HR.
Sol. A heated	1:16	1:128	1:128
Sol. A unheated	1:32	1:64	1:64

The same set-up was carried successively through the different temperatures. Saline controls were negative.

Protocol 2.—Lecithin, R serum agglutinated Type III cells almost completely after thirty minutes at 37° C. on the slide. The addition of lecithin later had no effect in releasing the clumps. The agglutination was likewise unaffected when the lecithin was mixed with the serum and cells before incubation. The observations were repeated with the same results at a temperature of 10° C. It was observed, however, that when lecithin had been added, the clumps of cells were rounder, cleaner cut, and less ragged.

Conclusions.—The isoagglutination is of high titer. It is not apparently affected by a temperature of 56° C. for thirty minutes. It is apparently slightly stronger at low temperatures. It is not reversible. It is not affected by lecithin. This blood contains no autohemolysin.

HETEROAGGLUTININS AND HETEROHEMOLYSINS

Protocol 3.—The following titrations were done with Solution A from unheated serum, no complement added. Dilutions 1:4 to 1:256 were made (see Table II).

TABLE II

HETEROAGGLUTININS AND HETEROHEMOLYSINS

	1 HR. 37° C.	1 HR. 10° C.	1 HR. 37° C.
	TITER	TITER	TITER
Sheep cells:			
Complete hemolysis	1:16	1:32	1:32
Partial hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:256	1:256
Horse cells:			
Complete hemolysis	1:16	1:16	1:16
Partial hemolysis	1:32	1:128	1:128
Agglutination	1:256+	1:256+	1:256+
Rabbit cells:			
Complete hemolysis	1:8	1:8	1:8
Partial hemolysis	1:16	1:16	1:16
Agglutination	1:256	1:256+	1:256+
Pig cells:			
Complete hemolysis	1:16	1:16	1:16
Partial hemolysis	1:64	1:64	1:128
Agglutination	--	1:256	1:256

The same set-up was carried successively through the different temperatures. Saline controls of all cells were negative for agglutination and hemolysis.

Agglutinations were repeated with sheep and pig cells in Solution A from heated serum. The titer for both was 1:128, one tube lower. This was not considered a significant difference.

Protocol 4.—Absorption: One cubic centimeter portions of Solution A from heated serum were absorbed with about 0.5 c.c. amounts of cell mush from sheep, guinea pig, horse, and rabbit at 37° C. for one hour with several shakings. After centrifuging the supernatant, serums were pipetted. Each of the four serums was titrated with fresh cells of all four species, making 16 titrations in all, the dilutions being from 1:4 to 1:64. Guinea pig complement, 0.5 c.c. of 1:30 dilution, was added to each tube (see Table III).

The same set-ups were carried successively through the different temperatures. Saline controls of all cells were negative for hemolysis and agglutination. Titers are those for partial hemolysis, as complete hemolysis did not occur in every series.

The sheep cell agglutinins were not completely absorbed. The titers are a little lower than in unheated serum. This, however, was not considered a significant difference due to heating (see note Protocol 3). It is possible that less complement was added here than was contained in the original serum.

With heated serum collected on another day the same absorptions were carried out and the agglutinations allowed to proceed on slides. The specific absorption of the agglutinins was clearly demonstrated. When lecithin was added to each of the mixtures, no change was noted in the results.

TABLE III

EFFECTS OF ABSORPTION

	1 HR. 37° C.	1 HR. 10° C.	1 HR. 37° C.
	TITER	TITER	TITER
Sheep Absorbed Serum:			
Sheep cells:			
Hemolysis	0	0	0
Agglutination	0	1:32	1:16
Pig cells:			
Hemolysis	0	1:8	1:8
Agglutination	0	1:8	0
Horse cells:			
Hemolysis	1:16	1:32	1:32
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	1:16	1:16	1:16
Agglutination	1:64	1:64	1:64
Pig Absorbed Serum:			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	1:32	1:32
Pig cells:			
Hemolysis	0	0	0
Agglutination	0	0	0
Horse cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	1:16	1:16	1:16
Agglutination	1:32	1:64	1:64
Horse Absorbed Serum:			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	--	--
Pig cells:			
Hemolysis	1:4	1:8	1:8
Agglutination	1:4	0	0
Horse cells:			
Hemolysis	0	0	0
Agglutination	0	0	0
Rabbit cells:			
Hemolysis	1:8	1:16	1:16
Agglutination	1:64	1:64	1:64
Rabbit Absorbed Serum:			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	1:64	1:64
Pig cells:			
Hemolysis	1:4	1:4	1:4
Agglutination	--	1:64	1:64
Horse cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	0	0	0
Agglutination	0	0	0

Conclusions.—Four differently absorbable and specific heteroagglutinins and heterohemolysins were demonstrated. The agglutinins were not affected by a temperature of 56° C. for thirty minutes. The titers of both agglutinins and hemolysins were increased slightly with lowering of the temperature. There was no clear evidence of reversal of the agglutination reaction. The titer of the hemolysins was not increased by warming after chilling. The heteroagglutinations were not affected by the addition of lecithin. The titers of the hemolysins were possibly within the normal human range.

AUTOHEMAGGLUTINATION

Protocol 5.—The following agglutinations were done on all of the three samples of R's blood, a Type II blood. The serum was unheated and unabsorbed. The results were identical in each instance (see Table IV).

TABLE IV

TEST FOR AUTOHEMAGGLUTINATION

MIXTURE	27-33° C. AGGLUTINATION	5-10° C. AGGLUTINATION
R serum + R cells	0*	+4
IV serum + IV cells	0	0
R serum + IV cells	0*	+4
IV serum + R cells	+4	+4†
Saline + R cells	0	0
Saline + IV cells	0	0

*Slight rouleau formation.
†Indicating isoagglutination.

A portion of the serum heated to 56° C. for thirty minutes gave the same results with R cells and IV cells. In the chilled mixtures the agglutination reversed after a few minutes at room temperature, leaving definite rouleaus in two of the specimens. In the other specimen the reversal was slight or absent. This will be commented upon later.

Protocol 6.—The effect of dilution on the autohemagglutinin: R serum diluted 1:3 with saline agglutinated R cells + 1 and IV cells + 2. The actual titer of this serum with R cells was 1:4.

Protocol 7.—Absorption of autohemagglutinin: Equal parts of R serum and R cell mush were placed at 5° C. for two hours and shaken several times. The mixture was centrifuged in a tube surrounded with crushed ice, and the serum quickly pipetted. This is designated Solution A. The remaining cells were not washed. To them was added an amount of warm saline equal to that of the serum removed, and the tube placed at 37° C. for thirty minutes. The mixture was shaken several times. It was then centrifuged in a water jacket at 40° C. and the supernatant fluid pipetted. This is designated Solution B.

The following mixtures were made on slides and agglutination observed after thirty minutes at 8° C. (see Table V).

The agglutination quickly reversed at room temperature (26° C.). There was no evidence of rouleau formation.

Protocol 8.—The effect of autohemagglutinin on heterologous cells: Of R serum heated to 56° C. for thirty minutes 0.5 c.c. portions were absorbed with

TABLE V

TEST FOR ABSORPTION OF AUTOHEMAGGLUTININ

	SOLUTION A AGGLUTINATION	SOLUTION B AGGLUTINATION
R cells	0	+4
IV cells	0	+4

approximately equal amounts of cell mush of guinea pig, rabbit, sheep and horse and Type III human cells at 37° C. for one hour. Mixtures were shaken several times. Mixtures were centrifuged at room temperature and serums pipetted. Mixtures were made on slides of each serum and fresh cells of the type used in the absorption (see Table VI).

TABLE VI

THE EFFECT OF AUTOHEMAGGLUTININ ON HETEROLOGOUS CELLS

	MIXTURE	37° C. AGGLUTINATION	5° C. AGGLUTINATION
Pig	absorbed serum + pig cells	0	+3
Rabbit	absorbed serum + rabbit cells	+2	+4
Sheep	absorbed serum + sheep cells	0	+4
Horse	absorbed serum + horse cells	0	+4
Type III	absorbed serum + Type III cells	0	+4

Saline controls of all cells were negative. The heteroagglutinin for rabbit cells was evidently not completely absorbed. The agglutination in all the other mixtures promptly reversed at room temperature (33° C.).

Protocol 9.—The effect of lecithin on autohemagglutination is shown in Table VII.

TABLE VII

EFFECT OF LECITHIN ON AUTOHEMAGGLUTINATION

MIXTURE	AGGLUTINATION
R serum + R cells + a drop of lecithin	+3
R serum + IV cells + a drop of lecithin	+3
Saline + R cells + a drop of lecithin	0
Saline + IV cells + a drop of lecithin	0
On slides at 10° C. for 30 minutes.	

Conclusions.—An autohemagglutinin was demonstrated for the subject's own cells, for Type IV human cells, for cells of guinea pig, sheep, horse, and probably of rabbit. The agglutinin was not effective at room temperature (28° C.) but was strongly effective under 10° C. It was not altered by a temperature of 56° C. for thirty minutes. Its reaction was completely reversible with increase in temperature. It was absorbable. It was not affected by lecithin. It appeared not to be reversible in the presence of other agglutinating factors such as heteroagglutinin and the rouleau-forming substance.

Two attempts were made to isolate the autoagglutinin in pure solution by washing in ice cold saline the cells remaining from Solution A and centrifuging in cups packed with brine. In both instances the temperature of the mixtures rose to between 15° and 20° C. before the process could be completed, and the

agglutinin was lost. This is mentioned to illustrate the great difficulty of working with this extremely thermolabile substance and the ease with which it may be overlooked in studies of serum.

Protocol 10.—Rouleau-forming substance: Rouleaus were observed in several tests of Solution A and in the mixtures of R serum with R and IV cells. The rouleau formation was never more than +2. In all these instances the addition of one or two 3 mm. platinum loopfuls of lecithin dispersed the rouleaus. This effect was clear-cut and complete, but did not occur until two or three minutes after the addition. The appearance of rouleaus was definitely more marked in chilled mixtures, and after such mixtures were warmed, and was usually absent in the same serum-cell combinations at room temperature and at 37° C. It is commonly stated that rouleaus are easily broken up by stirring, but this was not the case in these studies. The rouleau-forming substatnce was not found to be absorbable in any instance. Lecithin changed the shape of the erythrocytes by making them smaller and perfectly round in outline as viewed in one dimension.

COMMENT

Some observers consider autoagglutination an exaggeration of the rouleau-forming property. In the present instance the evidence is clearly to the effect that they are different, and that autohemagglutination is the result of a true agglutinin. The rouleau-forming property was not absorbable and was inhibited by lecithin, while the opposite was true of the autoagglutinin. In my experience clumping of red cells by the rouleau-forming property cannot always be distinguished from that of true agglutination by microscopic inspection, as advocated by Coca.[14] The action of lecithin appears to differentiate these two types of clumping quite clearly.

There is considerable resistance to the idea that an individual can develop an agglutinin against his own cells, in spite of numerous reports of human autohemagglutination. This agglutinin, however, is not specific in the sense that the isoagglutinins are, nor is it even species specific, but has the power of agglutinating the erythrocytes of many, possibly all, animals. An autoagglutinating property has been demonstrated in a large majority of the bloods of normal individuals by Landsteiner[1] and by Kettle.[4] Under certain conditions a powerful autoagglutinin appears, titers up to 1:250 having been reported.[5, 6, 7] It seems reasonable to suppose that these pathologic cold agglutinins are really Landsteiner's normal cold agglutinins increased in titer. The clinical states most often associated with these high titers are hemolytic jaundice, hemolytic anemia, some forms of cirrhosis of the liver, trypanosomiasis, repeated blood transfusions (in animals)[3, 5, 15, 16, 17] etc. In an experience of several hundred cross agglutinations designed to demonstrate autoagglutination I have found it frequently in patients requiring transfusions, and have seen sixteen posttransfusion reactions attributable to no other apparent cause. Most of these patients were sufferers from some hemolytic condition, chiefly from infections by hemolytic streptococci and staphylococci. Thus, in most of the diseases associated with autoagglutination, the destruction of red blood cells within the body seems to be a common factor.

This suggests that some product of the cell destruction acts as a stimulus (antigen) to the formation of the autohemagglutinin (panhemagglutinin).

The development of heterophile antibodies is usually attributed to the introduction into the animal body of the Forssman antigen. This idea is not controverted by the fact that the source of the antigen is not known (as in this case) since the antigen resides in many known, and probably in many unknown substances. Not all the facts of the present study, however, can be explained quite in this manner. To do so one would have to postulate the introduction of a Forssman type of antigen for horse, rabbit, and guinea pig erythrocytes as well as for those of the sheep, that is, an antigen with four different receptors, or else four different antigens simultaneously. The multiple production of heterophile antibodies from a single stimulus has been reported elsewhere. Deicher (quoted by Davidsohn[15]) found agglutinins for sheep, horse, rabbit, guinea pig, pig, and ox red cells in 90 of 102 human beings injected with horse (a few with sheep) serum. These agglutinins were specifically absorbable. Hanganutziu[16] found agglutinins for a series of animal red blood cells in about equal titer in 12 human beings injected with horse serum. These animals included sheep, horse, guinea pig, rabbit, veal, and pig. The suggestion that the heterophile sheep antibodies are Forssman stimulated, the others nonspecifically stimulated, hardly seems reasonable. A more plausible explanation, perhaps, is that of a powerful nonspecific stimulus to a general production of antibodies, the variety of these antibodies corresponding in a given case to such antigens as the cells have previously been "sensitized" to, or have the ability to produce naturally. Mino (quoted by Debenedetti) accounts for the appearance of panagglutinins on the basis of general stimulation. Kolmer[20] points to the fact of nonspecific stimulation of bacterial antibodies subsequent to an original specific stimulation, and suggests that the heterophile antigen may increase the production of natural sheep hemolysin nonspecifically. Bacterial agglutinins have been produced in rabbits by injection of heterophile antigen.[9] Thus the findings in the present study strongly suggest the existence of an unknown agent which stimulated the body nonspecifically to an excessive and apparently purposeless outpouring of antibodies. If this is found to be typical of all cases of infectious mononucleosis, then the disease is characterized, not so much by the presence of heterophile sheep antibodies, as by the appearance of many antibodies of various sorts.

CONCLUSIONS

A single human blood (from a convalescent case of infectious mononucleosis) was shown to contain, in addition to a high titer isoagglutinin, an autoagglutinin, four specific heteroagglutinins, four specific heterohemolysins, and a rouleau-forming property.

The characteristics of the several substances were demonstrated.

Some practical application of the findings to the problems of transfusion of blood have been discussed.

The study suggests the probability of the purposeless and excessive production of a number of antibodies simultaneously under certain conditions of nonspecific stimulation.

INFLUENZA VACCINE IN THE TREATMENT OF CHRONIC ENCEPHALITIS*

Results of a Study as to a Possible Relationship of the Pfeiffer Bacillus and Epidemic Encephalitis

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THE question of a possible relationship between influenza and epidemic encephalitis has been a mooted point since the pandemic of the former in 1918 and the appearance of the latter at approximately the same time. It was in that year that von Economo in Vienna and Netter, Hall and others, in adjacent countries, called attention to a group of cases which we have since come to know as epidemic encephalitis. In the wake of these diseases there began to appear a train of symptoms which have been designated as chronic encephalitis, the most common form of which is Parkinsonism.

In view of the fact that many of the patients presenting the picture of Parkinsonism gave no history of an antecedent acute encephalitis but had had the "grip," the "Spanish influenza," the "flu," or an upper respiratory infection, some investigators suspected a causal relationship between influenza and epidemic encephalitis. Crookshank,[1] one of the strongest advocates of such a relationship, was of the opinion that "The synthetic view, then, is that encephalitis lethargica represents a nervous form of influenza. . . ." He brought forth numerous historical references to substantiate his opinion and referred to the work of Tarozzi who also held the same view. Volpino and Racchiusa[2] advanced strong experimental evidence in support of such a relationship. Jaksch-Wartenhorsch[3] reported seventy-three cases of chronic encephalitis which he designated as postinfluenzal encephalopathy. Felsani[4] and Reichelt[5] did likewise in their series of cases. Zibordi,[6] in three children suffering from epidemic encephalitis, obtained a positive reaction to intradermal injections of influenza vaccine and to no other. Crafts,[7] too, leans strongly toward a relationship between the two diseases.

However, such investigators as Stern,[8] von Economo,[9] Flexner,[10] Jordan,[11] Strümpell[12] and others are of the opinion that epidemic encephalitis is an entirely distinct clinical entity with no direct relationship to influenza. In its survey of epidemic encephalitis the first report[13] of the Matheson Commission concluded, "It is apparent that the weight of opinion is either against the identity of influenza and epidemic encephalitis, or a direct relationship between the two diseases."

Many authors, nevertheless, consider favorably the possibility of some indirect relationship. Hall[14] expressed himself as being of this opinion. Flexner

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wrote, "Whether an attack of the first (influenza) acts to predispose to an attack of the second in the manner of the predisposing effects of influenza on bacterial affections of the respiratory tract, is another question awaiting close study." He asked further, "Could epidemic encephalitis be merely one manifestation, albeit a severe one, of epidemic influenza? That it is such a manifestation merely has been repeatedly asserted. The relationship has been as assiduously denied as upheld." Wimmer[15] evidently was of a similar opinion when he stated, "Should, now, the first attack be regarded as a true influenza, predisposing the soil for the encephalitic disease, as it has already been suggested by von Economo? Or should this and following attacks be considered as febrile encephalitic relapses. . . . ? Or could it possibly be a matter of reinfection?"

Kramer[16] is of the opinion that influenza is one of the factors, many of which are unknown, which are responsible for the epidemic spread of the sporadic cases of encephalitis. Zinsser[17] concluded that a great deal of the evidence supports the view that the coincidence of influenza and epidemic encephalitis may be causally related, one disease in some manner making the individual susceptible to the other.

One feels, therefore, that while the question of the possible relationship of epidemic encephalitis to influenza has been widely discussed and diametrically opposite opinions held by qualified investigators, one must agree with the conclusions of the second report[18] of the Matheson Commission that the question of the etiology of epidemic encephalitis is still unanswerable, and that the relationship of the two diseases requires more intensive study.

In this regard the work of Stewart and Evans[19] is of great interest. In 1930 these workers reported the results of their bacteriologic and clinical studies on patients with chronic encephalitis. They felt that no serious attempts had been made to discover whether there is a relationship between epidemic encephalitis and the Pfeiffer bacillus. To determine this, they studied 144 patients with Parkinsonism. These included patients who gave a history of an antecedent acute encephalitis or influenza. Nasopharyngeal cultures of these patients showed the presence of the Pfeiffer bacillus in 100 per cent of the cases. A control series of 122 ward and out-patients showed the Pfeiffer bacillus to be present in only 45 per cent of the patients. On the basis of these striking results, agglutination tests were made with the same patients. These tests were positive with the Hammett strain of the Pfeiffer bacillus in 100 per cent, the agglutination titers ranging from 1 to 20 to 1 to 60. As a check, the serums of these patients were found to be agglutinated in only three instances by other organisms. Of the 122 control patients only three febrile cases showed agglutination with the Pfeiffer bacillus.

Feeling that their work suggested a relationship between the Pfeiffer bacillus and epidemic encephalitis, the authors prepared a soluble antigen of the Hammett strain of the Pfeiffer bacillus. Sixty-two patients were injected intramuscularly with 0.1 c.c. of a 1 to 10,000 dilution of the soluble antigen every five to seven days for four doses. The dosage was later increased to 0.2, 0.3, 0.4 c.c. for four doses. Improvement, according to the authors, consisted in the reduction of rigidity. The patients felt less stiff, regained the use of the arms and

legs, the posture became more erect, the face more mobile and the speech less monotonous. No febrile reactions were noted. Stewart concluded that 78 per cent of her patients with chronic encephalitis showed definite decrease in rigidity. The tremors either were not affected or were often increased.

The results obtained by Stewart and Evans seemed to indicate a definite relationship between the Pfeiffer bacillus and epidemic encephalitis and, as such, are of great importance especially as we assume that this bacillus is the cause of influenza. We accordingly felt that their results warranted further investigation.

We have under our supervision approximately 100 patients with chronic encephalitis. Seventy of these are ward patients who have been observed from one to five years and whose activities are regulated. These patients are in a hospital for chronic diseases, a fact which predicates that they could not be cared for at home or could not adequately care for themselves. Some have been bedridden for many years. Most of them, however, are up and about with varying degrees of incapacitation. In the course of their residence in the hospital all have received at one time or another the tincture of stramonium in the dosage recommended by Jacobson and Epplen,[20] datura stramonium gr. iv t. i. d. or more often, hyoscine hydrobromide gr. 1/100 several times daily, atropine sulphate solution 0.5 per cent by mouth or by injection. Some of the patients have derived varying degrees of benefit from all or some of these drugs at one time or another. Because of the length of time they have been under our care and the control we have had over their medication, we have been able to establish a level for their physical state beyond which they did not seem able to go. Twenty-seven patients were seen in the Neurological Out-Patient Department of the Jewish Hospital. Most of them have been coming to the clinic for over three years at fairly regular intervals of two to three weeks. They, too, have received the various medications, except that none had been given any injections.

Nasopharyngeal cultures were made of all the patients. The medium used for cultivation was "chocolate agar" made by adding human red blood cells to heated nutrient agar of pH 7.4. The plates were incubated for twenty-four to forty-eight hours at 37° C. and the resulting growth examined with a hand lens for suspicious colonies. Subcultures of suspicious colonies were made on chocolate agar tubes and again on plain agar tubes. Organisms which showed the morphology of *B. influenzae* and which failed to grow on plain agar after two or three subcultures were considered to be of the *B. influenzae* group. Approximately three-fourths of the cultures were taken during the months from September, 1932, to January, 1933.

Of the 27 ambulatory cases from the out-patient clinic 11 or 40.7 per cent showed the presence of the influenza bacillus in the nasopharynx. A control group of 17 patients attending the same clinic for other neurologic conditions showed 5 or 29.4 per cent harboring the influenza bacillus. In the hospital 70 patients were studied. Only 13 or 18.5 per cent showed the presence of *B. influenzae* in the nasopharynx. Of 83 control cases from the same wards, 10 or 12.5 per cent were positive. Taken as a whole, out of a total of 97 patients with chronic encephalitis, 24 or 24.7 per cent showed the presence of the influenza

bacillus. Influenza was endemic in this vicinity during these months. Stewart and Evans, unfortunately, do not tell us what time of the year their cultures were taken.

Treatment with the Hammett strain of the Pfeiffer bacillus in the form of the soluble antigen was begun in 1932. At first the dosage recommended by Stewart was employed. When it was found that very little or no improvement was obtained with this dosage, all patients received 0.5 c.c. of the antigen weekly. As far as we could ascertain, there were no local or generalized reactions following the injections. Urine, blood, and temperature examinations during the course of the injections revealed nothing of significance. No untoward effects were ever observed from the injection of 0.5 c.c. of the antigen. At the beginning of the treatment many of the patients had been removed entirely from the medication they had been receiving for the past several months. Following the withdrawal of the medication it was found necessary to put a certain number back on their medication because of the development of difficulty in swallowing, marked weakness, rigidity, or for other symptoms common to this clinical syndrome.

Just before the injections were begun a physical status of each patient was determined by one of us (J. L. A.). This was compared with the condition of the patient at the completion of each series of four injections. Improvement was determined by questioning the patient and by noting his gait, his ability to get about and feed himself, the position of the body, the amount of rigidity in the arms, the intensity of the tremors, and the frequency of the oculogyric crises. The personal equation in evaluating the results was necessarily present, but since all examinations were done by the same individual, it was felt this would be minimized.

Approximately 125 patients with chronic encephalitis received injections of the soluble antigen of the Hammett strain of the Pfeiffer bacillus. Treatment in most cases consisted of a series of weekly injections of 0.5 c.c. of the antigen. In some cases the lack of any improvement and the hardship suffered by the patient by the withdrawal of all other medication caused us to cease using this form of treatment after one or two months. The majority of the patients, however, continued to receive the antigen for twelve to eighteen months.

CONCLUSIONS

It is often difficult to estimate the value of any method of treatment in the chronic stage of epidemic encephalitis. A change of medication is often likely to be attended by temporary improvement. In our series we found that those who were bedridden universally failed to receive any benefit from this or from any other form of treatment. A few of the patients, who were up and about and who were being benefited by large doses of drugs (hyoscine hydrobromide gr. 1/50 t. i. d.), maintained their physical level when they were receiving the injections by taking half the amount of the drug. It is difficult to explain this. At the end of treatment with the antigen over a period of eighteen months, we were unable to note any persistent improvement in any of the 125 patients, which

we could attribute to this form of treatment. Occasionally some of the patients thought they could walk with less rigidity, but they soon relapsed into their former state while continuing to receive the antigen.

Our results are in marked contrast to those obtained by Stewart and Evans. These workers reported improvement in 78 per cent of the patients treated by the same method. The material used by them and by us was manufactured and supplied by the same company.* We feel that our patients have been treated for a sufficient period of time to permit us to judge the efficiency of this form of treatment. Moreover, an attempt to emulate the laboratory work of Stewart and Evans in every detail resulted in a much lower percentage of positive cultures. Possibly the time of year when the cultures were taken might account for some of the discrepancy. In the treatment of the patients it occurred to us that possibly those patients who gave a history of having had the "flu" or the grip might react differently to the antigen of the Pfeiffer bacillus than those who gave a history of an antecedent acute encephalitis. However, we were unable to demonstrate such a difference. One must, therefore, conclude that as far as our results go, there is no relationship between chronic encephalitis and the Pfeiffer bacillus. If one assumes that this bacillus is the etiologic agent of influenza, then we feel that no direct relationship exists between influenza and epidemic encephalitis.

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*The antigen was supplied to us by the Eli Lilly Company for experimental purposes.

COMMENTS ON ANALYSES OF BLOOD CHEMISTRY OF CIRCA FIVE HUNDRED PATIENTS WITH COMMON SKIN DISEASES*

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ABOUT 500 patients were studied on the Out-Patient Service of Dr. Herman Goodman at the Stuyvesant Square, formerly the New York Skin and Cancer Hospital. Blood chemistry examinations were made of this group at the Research Laboratory of the Hospital. Charts were prepared graphically to present the findings, and our comments given here are based on the study of these charts.

SUGAR

The average blood sugar for 467 patients was 96.5 mg. per 100 c.c. We had 162 patients with acne (of whom 18 had seborrhea, 2 tinea, 1 eczema, and 1 furuncle). The average blood sugar for the acne group was 93.2 mg. which is slightly under the general average. The high of this acne group was 127.4 and the low 69.9. A group of 46 patients diagnosed as seborrheic dermatitis gave us an average of 94.4 mg. of sugar per 100 c.c. The high was 133.0 and the low was 76.4. Urticaria was also slightly below the general average, being 93.4 mg. for a group of 17 patients.

The highest average of sugar was among 23 patients with furunculosis. The high for this group was 218.2 and the low was 81.6. Patients with pruritus, 23 in number, gave an average of 102.4 mg. sugar. The high for this group was 229.4 and the low was 81.6 mg.

The other groups studied, dermatitis, eczema, erythema, pityriasis rosea, psoriasis and tinea, gave averages close to the general average of 96.5 mg. per 100 c.c.

We then relisted the cases, removing all patients with a blood sugar over 120 mg. per 100 c.c. The general average for 454 patients with blood sugar less than 120 was 94.4. The 82 patients with eczema gave an average blood sugar of 96.3 mg. per 100 c.c., which was well within the general average. Acne vulgaris (161 cases) gave an average of 93.0 mg. per 100 c.c. It has been recognized of late that this condition is not associated with the high blood sugar findings, once thought to be an accompaniment of the condition. Only one patient in this large group had a blood sugar over 120 mg. This patient had an associated seborrheic eczema with her acne and the blood sugar was 127.4 mg.

One patient of the group with furunculosis had a blood sugar of 218.2 mg. The average for the remaining 10 patients was 94.3 mg., the high was 110.0 and the low 81.6 mg. This would indicate that one of 11 patients with furunculosis was diabetic. No patient of the 19 with psoriasis had a blood sugar over 120

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mg. Three patients with tinea had very high blood sugar, 230.8, 194.6 and 187.4 mg., respectively. The average of the 39 tinea patients with less than 120 mg. of sugar was 94.0.

By and large then, the number of frank diabetics among patients seeking aid for skin conditions is probably no greater than in the general run of the same group, social, racial, etc., without skin affections. In all, seventeen patients had a blood sugar greater than 120 mg. per 100 c.c. We have already mentioned the patient with acne and seborrheic eczema (127.4); the patient with furunculosis (218.2); the three patients with tinea (230.8, 194.4, 187.4); and now list the remaining patients as: one with pruritus (229.4), four patients with eczema (198.6, 120.4, 120.4), one each with the following: stomatitis (200.0), tertiary syphilis (149.9), erythema (152.5), seborrheic dermatitis (133.0), herpes (130.2), dermatitis (124.6), and ichthyosis (120.4).

SODIUM CHLORIDE

The sodium chloride content of the blood was estimated in 466 patients. The general average was 471.6 mg. per 100 c.c. The highest average was found among 23 patients with pruritus, and it was 479.0 with a high of 515.8 and a low of 421.0 mg. per 100 c.c. The lowest average for sodium chloride was among the 11 patients with furunculosis and here it was 467.4 with a high of 483.8 and a low of 446.0 mg. per 100 c.c. The 162 patients with acne gave an average of 469.4 mg. per 100 c.c. The high for this acne group was 515.8 and the low was 420.1 mg. per 100 c.c.

The patients with eczema, 85 in number, gave an average blood sodium chloride of 472.4 mg. per 100 c.c. Nineteen psoriatics gave an average of 475.3 mg. of salt per 100 c.c. Other groups, as dermatitis, pityriasis rosea, tinea and urticaria, gave findings close to the general average.

By and for itself, the estimation of the salt and the analysis of the findings limited to the salt content gave us little practical information. On the other hand, a study of the report of the salt content of the blood with the accompanying sugar findings gave us some information. We grouped the blood chemistry findings of 514 patients and arranged them so as to compare the salt and sugar estimations. The average sodium chloride content was 475.5 mg. per 100 c.c. and the average blood sugar was 96.7 mg. per 100 c.c.

SALT AND SUGAR IN THE BLOOD

The exciting find we made was that as the blood chloride went up, the accompanying blood sugar went down. For example: we had 17 patients with blood chloride of less than 450.0 mg. per 100 c.c. The high of this select group was 441.8, the low was 408.5, and the average was 429.4. The sugar average for this group was 110.2 mg. per 100 c.c.

We next took a group of 25 patients with an average blood chloride of 446.5 mg. per 100 c.c. The high for this group was 450.1 and the low was 441.8. The blood sugar for this group was 103.2 mg. per 100 c.c. We next selected the cases in groups of fifty with the salt content graduated upward. As the salt averages went up, the sugar content averages went down. It was

not exactly mathematical, but when we reached the average blood chloride of 498.4 for a group of fifty patients with high 507.5 and low 495.0, we found that the blood sugar was 92.9 mg. per 100 c.c.

Now, we thought, if the blood sugar apparently goes down as the salt goes up, what will the figures show if taken from the findings of the sugar instead of the salt? So we rearranged our cases once more. And we find that as the average blood sugar goes down, the average blood chloride goes up. For example, we had 17 patients with blood sugar over 120 mg. per 100 c.c. The blood chloride for this selected group was 455.1 mg. per 100 c.c. The next group of 25 patients, with blood sugar between 110 and 120 mg. per 100 c.c., had an accompanying blood chloride of 475.0 mg. per 100 c.c. Again, we found that the sliding scale was not exactly mathematical, but if we jumped to a group of 50 patients with an average blood sugar of 83.4 (high 85.5 and low 80.2), we find that the blood chloride was 479.8 mg. per 100 c.c.

We check back with our list of patients with high blood sugar and find that the patient with pruritus who had 229.4 mg. of sugar per 100 c.c. of blood had only 421.0 mg. of sodium chloride. The patient with furunculosis who had 218.2 mg. of sugar per 100 c.c. of blood had 446.0 mg. of salt per 100 c.c. The patient with stomatitis with a blood sugar of 200 had 450 mg. of salt. A patient with eczema who had 198 mg. of sugar had 450.1 of salt. One patient with tinea who had a blood sugar of 187.4 gave a blood chloride of 433.5. Of course, this comparison was not always mathematical. If it had been, we would be concerned that the figures were not factual. For example, a patient with tinea who had a blood sugar of 230.8 had a blood salt of 454.3. Another patient with tinea had a blood sugar of 194.4 and a blood chloride of 462.7 mg. per 100 c.c. In both these instances, the blood sugar was much over the average of 96.5 and in turn the blood chloride was much below the average of 471.6. In the above list, we have accounted for the highest 10 of the 17 patients with blood sugar of more than 120 mg. per 100 c.c.

We would turn next to a tabulation of 14 patients who gave us a sodium chloride content of less than 441.0 mg. per 100 c.c. There were three patients of these 14 who had blood sugar of more than 120 mg. per 100 c.c., and two other patients close to it (119.0 and 117.7).

The other nine patients had a low salt content without accompanying high blood sugar; four of these had 437.7 mg. of salt per 100 c.c. The lowest salt content was in a patient with seborrheic eczema whose blood sugar was 81.6 mg. We find listed a patient with pemphigus showing salt content of 437.7 and a sugar of 92.0 mg. per 100 c.c. This is of some interest in the proposed etiology of this strange disease.

Another point of interest is that six patients in this list of low salt content (less than 441.0 mg. per 100 c.c.) were diagnosed acne vulgaris. Not one had a blood sugar above 120 mg. per 100 c.c. We did not recognize the importance of this until much later, and hence are unable to report exactly what clinical type of acne vulgaris is represented by these six patients.

UREA NITROGEN

We learned nothing of startling import by our analyses of the findings of urea nitrogen in the blood of the 466 patients studied. The general average

was 13.5 mg. per 100 c.c. All the groups studied, acne, dermatitis, eczema, erythema, furunculosis, pityriasis rosea, pruritus, psoriasis, tinea, and urticaria were well within this general average. Furunculosis, for example, was low with 13.0 and pruritus was high with 14.5.

URIC ACID

We cannot report anything unusual in the findings for uric acid. The general average for the 466 patients tested was 2.55 mg. per 100 c.c. The low was 2.07 for pityriasis rosea. The high was 2.90 for psoriasis.

INFLUENCE OF AGE OF PATIENT ON BLOOD FINDINGS

It seemed worth while to recast our findings according to the age of the patient as well as according to the different ingredients of the blood studied.

INFLUENCE OF AGE OF PATIENT ON BLOOD SUGAR

We estimated the blood sugar on 514 patients and found the general average to be 96.7 mg. per 100 c.c. All the groups less than forty years of age averaged less than this general average. For example, 111 patients were between twenty-one and twenty-five years of age. The average blood sugar for this group was 92.1. The group between forty-one and fifty years had 63 patients. The average for this group was 108.4. All the groups of patients more than forty years of age had blood sugar averages more than 105.6 mg. per 100 c.c.

At first sight then, it would seem that what we are stating is that there must be more diabetics among the higher age groups. But we recast our findings once again and remove all blood sugar above 120 mg. per 100 c.c. In general, the average drops from 96.7 to 94.7 as the 17 patients with blood sugar over 120 are deleted. But the swing upward of the blood sugar average with increasing age is still noted. It happened that the group of 111 patients between twenty-one and twenty-five had no blood sugar above 120 so its average remains 92.1. The age group forty-one to fifty lost 8 patients with blood sugar above 120. The average for the 55 patients remaining was 101.3 mg. per 100 c.c., the highest recorded average. The patients more than forty-one had an average of 100 mg. per 100 c.c., which is higher than the general average for all age groups of 94.7 mg. per 100 c.c.

INFLUENCE OF AGE ON SODIUM CHLORIDE IN THE BLOOD

The general average of sodium chloride in the blood was 475.5 for the 514 patients studied. The averages for the various groups held quite close to this general average. The highest average for the age groups was 478.7 for the ages thirty-one to forty. The lowest salt average was 468.7 for the age group fifty-one to sixty. In a way, the conduct of the salt holds to our former findings, for the older people, that is above fifty-one years old, averaged the lowest salt and the highest sugar findings. Again this was not mathematical, and we are glad that it was not so for it would smack too much of window dressing.

INFLUENCE OF AGE ON UREA NITROGEN IN THE BLOOD

The general average of urea nitrogen for 502 patients was 13.6 mg. per 100 c.c. The lowest age group was that between twenty-one and twenty-five.

There were 111 patients in this group and the urea nitrogen averaged 12.8 mg. per 100 c.c. The highest average was for the small group of 15 patients more than sixty years of age with an average of 15.7. But the next highest was for the youths of our series, patients under fifteen years of age, of whom there were 22 who had an average of 14.8 mg. of urea nitrogen per 100 c.c. of blood.

INFLUENCE OF AGE ON URIC ACID IN THE BLOOD

The general average of uric acid for 500 patients was 2.62 mg. per 100 c.c. The lowest group was between sixteen and twenty years of age. There were 82 patients in this group and the average was 2.39 mg. of uric acid per 100 c.c. The highest average was in the group of 45 patients between the ages of fifty-one and sixty. The uric acid average was 2.93 mg. per 100 c.c.

SUMMARY

We have commented upon the results of blood chemistry studies made on about 500 patients with various skin disorders. We note that blood sugar is high in furunculosis as an average because one patient was a frank diabetic with a very high blood sugar. We offer, then, the process of consideration of our average finding with all frank diabetics removed. Then, the sugar findings are without any particular significance by themselves.

The analyses of the salt findings were not significant by themselves, either. But we disclosed a relationship between salt and sugar which we thought very significant; namely, that by and large as the sugar averages went down, the salt averages went up. This relationship, the antagonism between sugar and salt, was not absolute. It was fairly constant. The converse was also noted: as the average salt content of the blood increases, the average blood sugar decreases. We do not offer any reason for this reciprocal arrangement. We are certain that it is not fiction nor bookkeeping.

On the basis of the findings of antagonism between sugar and salt in the blood, and the accidental find that saline intravenously relieved a pustular acne (which we first considered an acne due to ingestion of bromides), we have experimented with salt solution intravenously and locally into pustules, with gratifying results. The further possibilities of this form of therapy are suggested.

We found nothing exciting in the blood urea nitrogen or uric acid among our patients.

We did find that the content of sugar gradually went higher with increasing years, and that this was true whether we deleted frank diabetics or left them in the analysis. Older patients retain sugar, urea nitrogen, and uric acid. They do not retain more salt, but tend, as the sugar goes up, to have less than the average saline content of the blood.

We believe that this method of presenting blood findings for a series of about 500 patients, seeking aid for dermatologic ailments, has not been previously presented.

18 East 89th Street

EXPERIMENTAL STUDIES IN SO-CALLED AGRANULOCYTIC ANGINA*

Effects of Toxic Products of Certain Bacteria Recovered from the Stool and Blood of the Human Being Upon the Leucocytes of Animals

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THIS unusual clinical entity was first described by Schultz[1] in 1922 and called by him agranulocytic angina. The accuracy of the name had been questioned in various reports, and other names deemed more suitable have been applied. The disease has been called angina agranulocytic (Friedman), mucositis necroticans agranulocytica (Weiss), granulocytopenia (David), and malignant neutropenia (Schilling). A solution of such a discussion may perhaps be reached by terming this condition "the Schultz syndrome," especially as thus far the indications are that its causation is of a protean rather than a specific nature. The leucocytic picture present is generally a granulocytopenic leucopenia, whereas the associated clinical and other laboratory findings may at times vary. A destructive throat lesion is, however, usually present. Replete and recent compilations of the literature are given by Kracke[2] and in the editorial symposium section of the *International Medical Digest*.[3] The study of the bone marrow and a complete review of the literature on this phase of the disease recently have been presented by Jaffé.[4]

In experiments relating to the cause of the Schultz syndrome various observations have been reported. Denn's[5] has shown that when various virulent microorganisms are enclosed in parchment capsules and introduced into the peritoneal cavity of rabbits, in some instances reduction of the total leucocytes and granulocytes is produced by the absorption of the permeating toxins. The microorganisms employed by him were not derived from the human disease, excepting a *B. proteus* which did not cause any marked changes. Kracke[6] reports that various microorganisms recovered from throat lesions and other areas fail to produce granulocytic neutropenia when injected into animals. He produced the blood picture and, in some instances, throat lesions by the injection of benzene with olive oil. It had been previously shown by Selling both clinically[7] and experimentally[8] that benzene is capable of producing in certain patients a granulocytopenia with purpura and hemorrhages, and in the experimental animal, reduction of the leucocytes to 200 cells per c.mm. with but little alteration of the erythrocytes. In this

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connection, because of a supposed benzene content, much interest has recently arisen as to the probable causal relationship of the various barbital derivatives. It must be realized, however, that some of these drugs considered as causes actually contain no benzene factor, and furthermore that other drugs of the benzene group in very general use are not incriminated.

MATERIALS

Our materials were procured from a fatal chronic case occurring in a child, eleven years old, in whom the primary throat lesion, diagnosed as Vincent's angina, had disappeared but a marked blood dyscrasia was persistent. The patient presented boils and two large abscesses and revealed a positive *Staphylococcus aureus* blood culture several days prior to death.

As far as ascertained by us, the possible causal relationship of certain of the intestinal flora of such cases has not been investigated. We accordingly carried out a bacteriologic investigation of the stool specimens, and recovered cultures of *B. enteritidis*, *B. welchii*, and *Streptococcus hemolyticus*. In the experiments herein reported, these cultures obtained from the stools and also the culture of *Staphylococcus aureus*, procured from the blood culture together with toxic products obtained from these isolations, were employed. These were injected by various routes into animals to ascertain their effects, especially upon leucocytes. Seventy-nine guinea pigs and sixteen rabbits were used. The leucocytic counts of rabbits as found by other observers[9, 10, 11] and ourselves[12] reveal total counts of from 8,000 to 10,657 cells per c.mm. and a differential granulocytic count of 35.4 per cent to 54.0 per cent. These counts in the guinea pig show the usual established variation of from 6,000 to 12,000 leucocytes per c.mm. and granulocytes of approximately 35 per cent.[13]

EXPERIMENTAL

B. enteritidis.—Six guinea pigs were injected, two subcutaneously, two intraperitoneally, and two intracardially with 1 c.c. each of a saline suspension, containing approximately 2.5 billion bacteria per cubic centimeter of this strain of *B. enteritidis* and all died in from twenty-four to seventy-two hours. Although repeated counts were made even up to shortly before death, no evidences of reduction of the total leucocytes or granulocytic counts were produced but on the contrary a neutrophilic leucocytosis prevailed.

Three guinea pigs were fed daily with oats contaminated with the stools of the human being containing *B. enteritidis* and over the food of 3 additional guinea pigs, suspensions of the recovered culture of this bacillus were poured. After six weeks of observation, including repeated leucocytic counts, no appreciable effects of these feedings were noted.

Four guinea pigs were injected with the filtrate of broth cultures of the *B. enteritidis*, two intracardially and two intraperitoneally. The resultant leucocytic counts showed a total and granulocytic increase.

Since such direct introductions of the *B. enteritidis* and culture filtrates failed to produce granulocytic leucopenia, it was decided to employ the toxic products that might be procured through the in vivo method as used by one

of us (Harris) in the experimental production of the lesions of typhoid fever,[14, 15] with a concurrent leucopenia. In this method fatal peritonitis is primarily produced in the guinea pig and the exudative material and washings of the peritoneal cavity are collected and filtered through a Berkefeld or Seitz-Wertz filter. The purpose of this procedure is to obtain the toxic factors that may result from the invader and host conflict. The supply pigs receive 2 c.c. intraperitoneally of a heavy suspension of the living *B. enteritidis* suspension. Death from the induced peritonitis occurs in twelve to twenty-

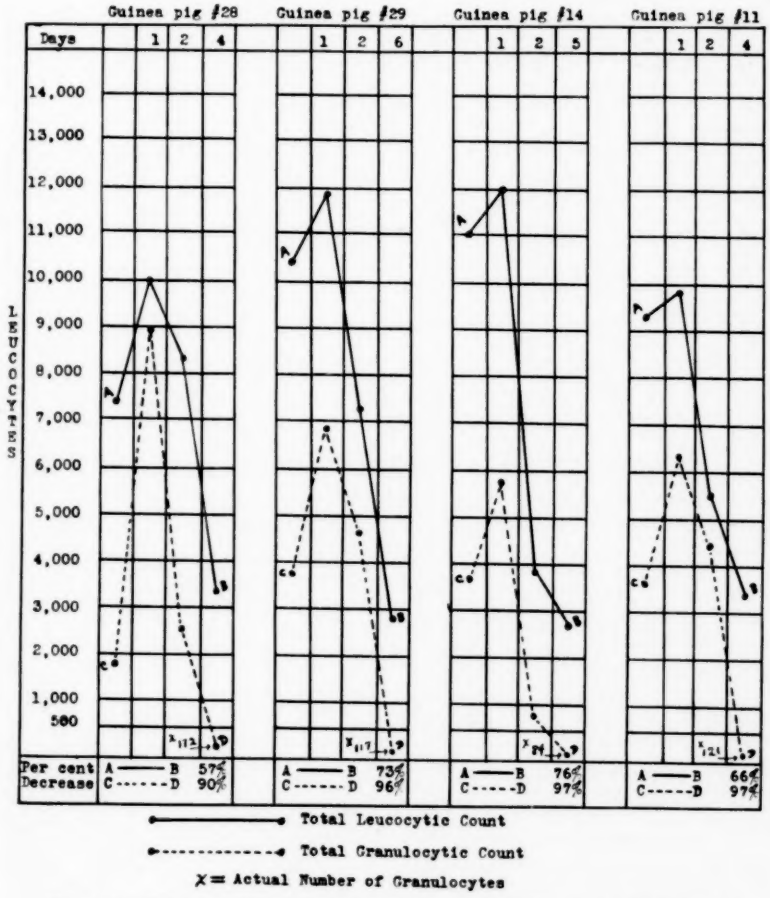

Chart 1.—Effects of toxic products of *B. enteritidis* upon leucocytes.

four hours. Fifteen guinea pigs were injected with the toxic materials procured through this method. The toxic Seitz-Wertz filtrate was administered in doses of from 1 to 5 c.c. subcutaneously, intraperitoneally, and intracardially to different groups of animals. The intraperitoneal and intracardial routes and the larger doses were more effective. In all animals injected with the filtrate, a distinct lowering of the total leucocytic counts with a proportionately greater reduction of the granulocytes was observed. The period at which the greatest decline occurred averaged seventy-two hours with ordinarily an increment in the first twenty-four hours. As a series, the intra-

cardiac route yielded the most marked leucopenia and granulocytopenia. Representative changes produced in the leucocytic counts of four animals thus inoculated are shown in Chart 1. Reductions of from 57 per cent to 76 per cent of the total white cell count and of from 90 per cent to 97 per cent of the granulocytes are demonstrated. Four guinea pigs were injected intracardially with toxic peritoneal filtrate procured from a stock strain of *B. enteritidis* and the inhibitory effect upon the granulocytes and leucocytes

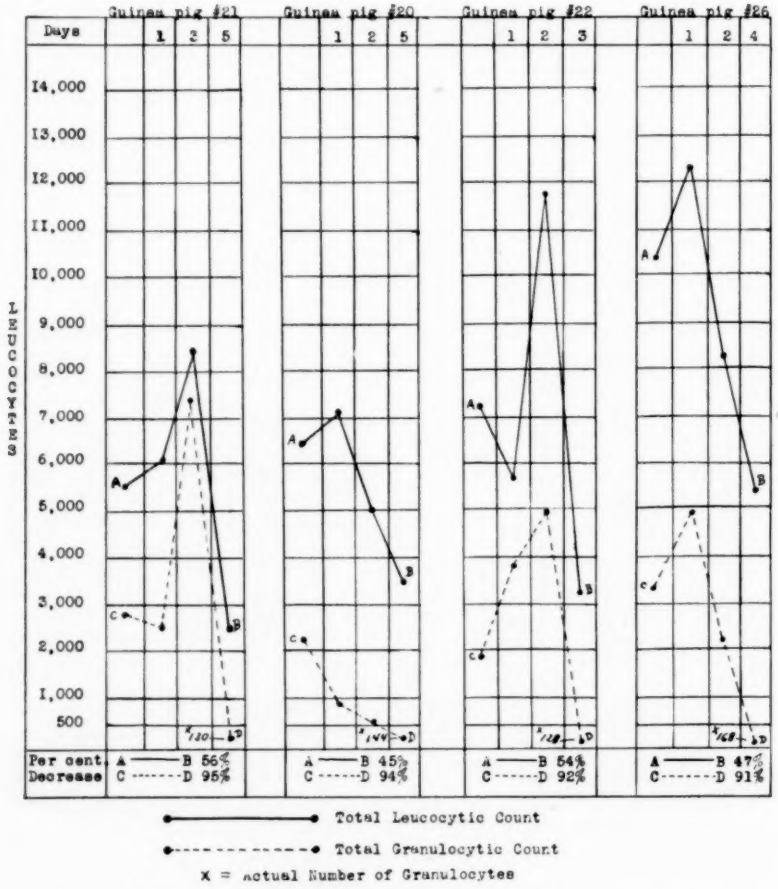

Chart 2.—Effects of toxic products of *B. typhosus* upon leucocytes.

as a whole was much less marked. This result is attributed to the fact that this culture had been carried in stock for over four years.

For comparison, an old stock culture of *B. typhosus* that was known to depress leucocytes by its in vivo toxic products, was employed. Twelve guinea pigs were treated in the same manner with toxic peritoneal exudate filtrates of *B. typhosus* peritonitis as were those with the enteritidis and, in general, similar results were obtained. The results of four guinea pigs injected intracardially with this material are shown in Chart 2. The culture used in these injections had been in artificial cultivation for about five years. When this

culture was primarily recovered from the patient and further passed through several guinea pigs, the resultant leucopenia was much more marked and the gross and histopathology of typhoid fever were produced.[15]

B. welchii.—Upon the first series of animals consisting of six full-grown rabbits, the recovered cultures of *B. welchii* were employed in the following manner: The whey portion of litmus milk cultures was filtered through a Seitz-Wertz filter and the toxic filtrate retained. It is to be appreciated that various protein products aside from the bacterial toxin were contained in the

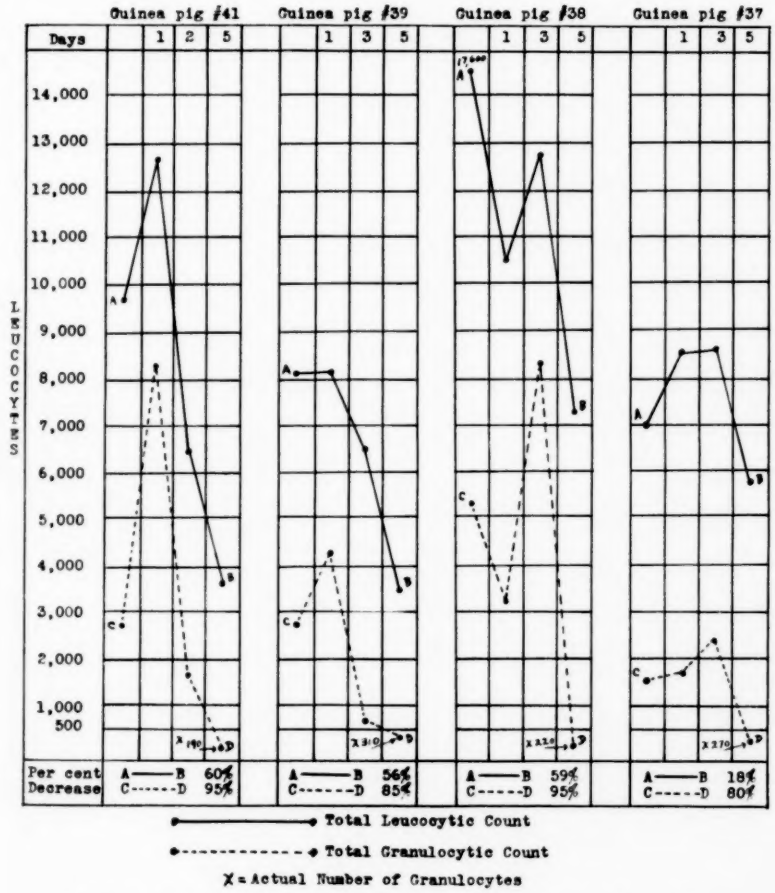

Chart 3.—Effects of toxic products of *B. welchii* upon leucocytes.

filtrate. This filtrate in amounts of from 2 to 10 c.c. was introduced daily for two weeks into the stomach through a tube. No effects upon the general well-being of these animals or the leucocytic counts were noted. The living culture of this microorganism was administered through a tube into the stomach of four rabbits. These animals, likewise, showed no changes in their physical condition or in the frequent leucocytic observations that were made.

The toxic filtrate of *B. welchii* was next administered into two rabbits intravenously and nine guinea pigs intracardially. Each of the two rabbits was given two doses intravenously of 1 c.c. at six-day intervals. The total

leucocytic count was primarily increased following these injections with a corresponding increase of the granulocytes, but after seventy-two hours the total count returned to normal. The granulocytes, however, were reduced from an average of approximately 40 per cent to amounts as low as 5 per cent following a primary rise, but later returned to normal. The guinea pigs injected intracardially with this toxic filtrate in doses of more than 0.5 c.c. usually succumbed within twenty-four hours. In the animals inoculated with 0.25 to 0.5 c.c. the leucocytic and granulocytic curves were the same as those of

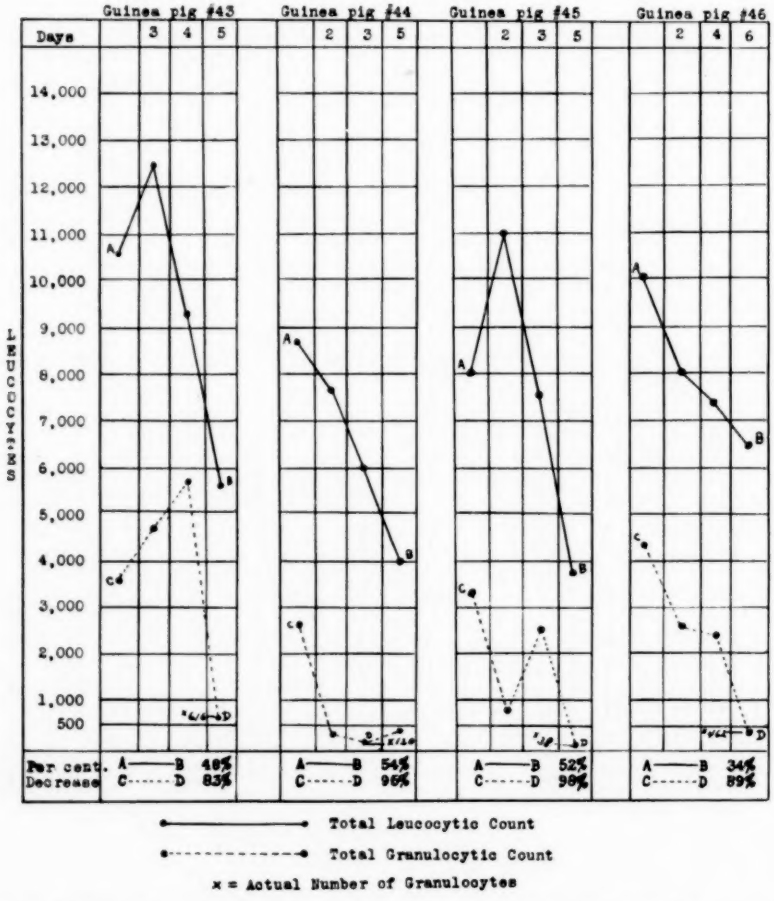

Chart 4.—Effects of toxic products of *Streptococcus hemolyticus* upon leucocytes.

the rabbit, i.e., primary rise of both with subsequent return of the total count but diminution of the granulocytes to between 5 and 8 per cent. Four guinea pigs were injected intraperitoneally with 3 to 5 c.c. of the welchii filtrate, and these animals yielded in certain instances marked reductions of both the total leucocytic and granulocytic cells. The effects produced upon the leucocytes are shown in Chart 3. Reductions of the total white cell counts of from 18 per cent to 60 per cent are seen, whereas the granulocytic reductions were as much as 95 per cent.

Streptococcus Hemolyticus.—The inoculation of this culture subcutaneously, intraperitoneally and intracardially yielded neutrophilic leucocytosis. Filtrates of broth cultures introduced into four guinea pigs did not reduce the leucocytes or granulocytes. In vivo prepared toxic products were obtained by intraperitoneal injections of this microorganism into the guinea pig. As it was only slightly pathogenic for this animal, two injections of heavy suspensions were given at seventy-two-hour intervals when peritonitis and death ensued. The Seitz-Wertz filtrate of the peritoneal exudate and washings was

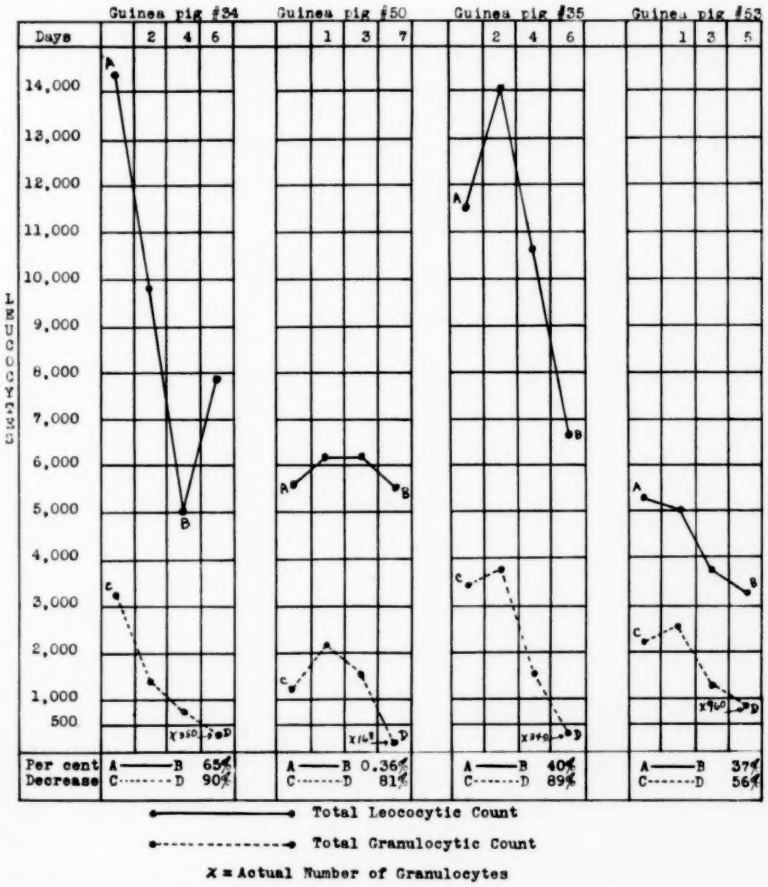

Chart 5.—Effects of toxic products of *Staphylococcus aureus* upon leucocytes.

injected intraperitoneally into two guinea pigs, Nos. 45 and 46, in doses of 5 c.c. and intracardially into guinea pigs Nos. 43 and 44, each receiving 3 c.c. Considerable reduction of the total leucocytic count with marked reduction of the granulocytes was produced as shown in Chart 4. Again, it is shown that both marked reductions of the total leucocytes (54 per cent) and granulocytes (98 per cent) were produced.

Staphylococcus Aureus.—Injections of suspension of the culture of *Staphylococcus aureus* subcutaneously into two guinea pigs and intraperitoneally into two other such animals produced neutrophilic leucocytosis. Rabbits 2,

3, and 5 were inoculated in the following manner: Rabbit 2 received 2 c.c. of the living suspension in the peritonsillar structures which eventually produced a local necrosis in this area. Frequent counts revealed only a neutrophilic leucocytosis. In Rabbit 3 the upper tracheal mucosa was scarified with the hypodermic needle and 1 c.c. was injected in and about the eroded area. Neutrophilic leucocytosis ensued. Rabbit 5 was given 5 c.c. of melted agar subcutaneously. After the agar became semisolid, 1 c.c. of the culture

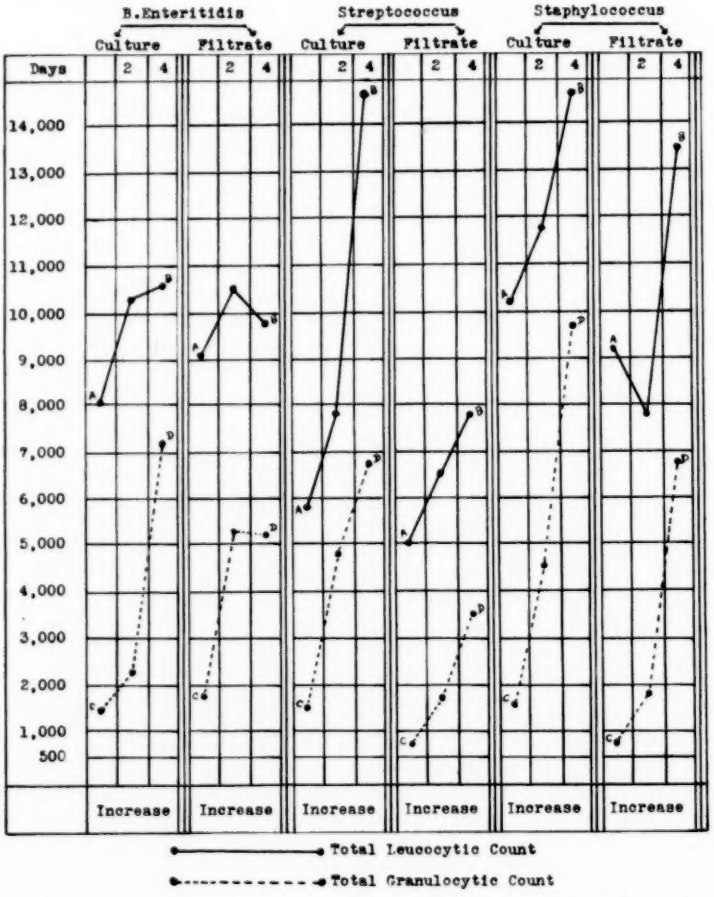

Chart 6.—Control animals injected with living culture or culture filtrate.

of *Staphylococcus aureus* was introduced into the mass. Only a slight rise of the total leucocytes and granulocytic cells was noted upon repeated counts.

Four guinea pigs were injected with the filtrate of broth cultures of this staphylococcus, two intracardially and two interperitoneally. These animals revealed only neutrophilic leucocytosis.

An in vivo toxic filtrate prepared as in the previous instances was obtained from this staphylococcus and injected intraperitoneally and intracardially in doses of 3 c.c. into six guinea pigs, three for each route. Some reduction of the total leucocytic count and marked reduction of the granulocytes were produced (see Chart 5). It is of interest to note that one of these

animals developed an erythroblastic anemia, indicating a disturbance of this portion of the hemopoietic system as well as that of the myeloblastic centers.

As has been heretofore stated, the direct injections of the employed cultures of *B. enteritidis*, streptococcus and staphylococcus and their broth culture filtrates failed to produce granulocytopenia. In Chart 6 is shown a number of these animals injected with the culture and culture filtrates which serve as controls in comparison with the animals injected with the in vivo prepared toxin.

In general, it may be stated that repetition of the injections of in vivo prepared toxin into the same animals produced a duplication of the results described. After several such injections, the majority of the animals sickened and died. In these animals pneumococci and terminal invaders were obtained from hearts' blood.

Smear preparations of the bone marrow of those animals demonstrating marked granulocytic depression showed, as a whole, degenerative changes in the nuclear portion of the myeloid cells. Different findings have been noted by various observers, a compilation of which has been set forth by Jaffé.[4]

DISCUSSION

From the results presented herein and the work of others, it can be appreciated that various microorganismal poisons will produce the marked leucocytic changes occurring in the Schultz syndrome or so-called agranulocytic angina as found in man. The continuation of such leucocytic depressions eventually leads to a morbidity of the animal with lowered resistance and secondary infection as frequently noted in the human being.

Whether such parallelisms represent a true reproduction of the human disease is naturally subject to question and, no doubt, will remain so unless a specific causal agent is later discovered. In this connection, it is difficult to understand, if a heterologous group of toxic factors are responsible for the disease, why it does not occur more frequently. It might be surmised that this rather unusual phase of certain toxins occurs infrequently, or, as has been suggested,[16] a rather rare allergic phenomenon is displayed.

In the human being, it is rather remarkable that a secondary invasion of *Staphylococcus aureus* occurs so frequently unless the lowered neutrophilic resistance and wide distribution of this microorganism is considered responsible. In connection with such staphylococcal invasions occurring both locally and at times systemically, it is of note that such a pyogen with actual production of pus or an accumulation of neutrophilic granulocytes should be accompanied by a systemic granulocytic depression or at times absence of such cells in the circulating blood stream. Perhaps the "shifting of the vascular bed" as indicated by Garrey[17] might explain their source. Kracke,[2] however, has demonstrated that the blood removed from the veins in this disease demonstrates the same marked leucocytic and granulocytic depression.

Dennis[5] considers that the absorption of toxic material without bacterial invasion into the tissues may cause this condition. He, therefore, employed the intraperitoneal parchment capsules containing the microorganism to dem-

onstrate this possibility. In the present experiments, with the exception of the *B. welchii* toxin, the liberated toxic moieties were obtained from filtrates of the peritonitis exudates. It is considered that in this in vivo encounter of microorganisms and host there is liberated a toxic substance that is not ordinarily obtained in vitro. Such in vivo toxins can be readily obtained, and while their activity when injected is transitory, repeated injections will serve as a sufficiently continuous source.

CONCLUSIONS

1. From the observations made herein, it is evident that marked depression of the total and granulocytic leucocytic counts can readily be produced by the toxic moieties of various bacteria. The toxin of bacteria of this disease, obtained from other sources than that of the human being, will likewise cause such reductions. Recent isolations in which the virulence is exalted produce more distinctive results.

2. For the bacteria herein employed, a toxic moiety obtained only from an in vivo source is effective, with the exception of *B. welchii*. Repetitions of such toxic injections into animals reduce their resistance, and they become sick and die, frequently with terminal infections.

3. Relative to the etiology of agranulocytic angina or the Schultz syndrome, these experiments would indicate that the causation is of a protean or varied nature.

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A STUDY OF THE COMPARATIVE VALUE OF THE LÖWENSTEIN METHOD AND THE DIRECT CULTURE OF WHOLE BLOOD IN DETECTING TUBERCLE BACILLI IN THE BLOOD STREAM*

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TUBERCULOUS bacillemia has been the subject of a great deal of study, especially since Löwenstein and his associates[1 a, b, c, d] reported a high percentage of positive results not only in various tuberculous infections, but also in apparently nontuberculous conditions such as rheumatic fever, polyarthritis, and dementia precox. Löwenstein devised a special method of culturing the blood for tubercle bacilli[2] in which the hemoglobin is removed from the blood before the specimen is cultured. He stressed the importance of completely removing the hemoglobin as he believed that it interferes with the growth of the tubercle bacilli. Many have attempted to confirm Löwenstein's work but only a few reported successful results. Most of the workers were able to obtain only a comparatively small percentage of positive cultures in tuberculous cases, and some had entirely negative results or obtained a few saprophytic acid-fast cultures.[3 to 15] According to Löwenstein, however, this failure to obtain positive results may have been due in part to incomplete removal of the hemoglobin.[1 d]

One of us[16] found in connection with other investigations that human and bovine tubercle bacilli grew freely and rapidly on the Bordet-Gengou medium which contained about 30 per cent of horse blood. Also that human tubercle bacilli grew freely when the whole blood of infected rabbits was cultured on the Bordet-Gengou and on egg medium without any preliminary treatment.[17] Dreyer and Vollum[18] obtained positive blood cultures from rabbits and guinea pigs by inoculating whole blood into plain or glycerin bouillon. Alexander[19] obtained positive cultures from tuberculous mice by growing the whole blood on the Bordet-Gengou medium.

Since the hemoglobin of animal blood apparently did not interfere with the growth of tubercle bacilli, it seemed desirable to determine whether human blood differs in this respect from animal blood. If in the search for tubercle bacilli whole blood could be cultured directly, it would be very much simpler and consume much less time than culturing according to Löwenstein's method. With this object in view we carried out the following investigation:

Specimens of blood were obtained from tuberculous patients at the Sea View Hospital. The amount of blood cultured varied between 4 and 14 c.c. depending upon the ease with which the blood was obtained. In order to make

*The "Whole Blood Culture" was carried out at the Research Laboratory of the New York City Department of Health under Director Dr. Wm. H. Park, and the "Löwenstein Culture" was carried out at the Sea View Hospital Pediatric Department under Dr. Bela Schick.

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the two methods directly comparable each specimen was divided into two equal parts. One part was mixed with sterile 0.4 c.c. of 10 per cent sodium citrate to prevent coagulation and was used for the direct culture, and the other part was cultured by the Löwenstein method.[2]

WHOLE BLOOD CULTURE METHOD

As soon as the citrated blood was received at the laboratory, it was inoculated by means of a pipette on the surface of Bordet-Gengou and of Congo red egg* mediums in Petri plates, using 6 to 10 plates for each specimen. Then to each plate was added about 1 c.c. of sterile distilled water in order to facilitate the spread of the blood thus hemolyzed, and the plates were tilted back and forth until the blood was evenly distributed over the whole surface. They were then incubated at 37.5 to 38° C. for two to three days or until the excess moisture had evaporated. Care was taken not to allow the plates to become too dry. After this preliminary incubation the plates were sealed with three-fourth-inch Scotch photographic tape and incubated for two months. During the period of incubation the cultures were examined by means of a low power microscope once a week to detect an early growth. Any growth that even remotely resembled colonies of tubercle bacilli was examined by smear and in some instances by subculture.

LÖWENSTEIN'S METHOD

The blood was mixed with 2 to 3 c.c. of sterile 10 per cent sodium citrate in centrifuge tubes of 50 c.c. volume and well shaken to prevent clotting. About 30 c.c. of sterile distilled water was added; the contents were shaken and centrifuged for twenty minutes at about 2,000 revolutions per minute. The supernatant fluid was decanted; the sediment was well shaken and again washed with about 30 c.c. of sterile water, and centrifuged for twenty minutes at about 2,000 revolutions per minute. The supernatant fluid was decanted, leaving behind a small grayish sediment. The sediment was thoroughly broken up with a pipette (3 mm. bore) into which all the material was drawn and then spread over the surface of 3 or 4 tubes of Löwenstein's medium. The tubes were well sealed at once with sealing wax, and allowed to lie horizontally for twenty-four hours. Then the tubes were placed upright in the incubator at 38° C. for two months. If no growth was macroscopically visible at this time, a smear was made of the edges, bottom, and surface of the medium. If the result of the microscopic examination was negative, the surface of the tubes was scraped into a small amount of distilled water. This was centrifuged for one-half hour and the sediment again examined by smear. In all microscopic examinations, the Ziehl-Neelsen method of staining was used and the whole surface of the smear was examined by means of a mechanical stage.

We have examined in this manner 98 specimens of blood from 63 tuberculous patients and 2 specimens from a case of actinomycosis of the jaw. The results obtained are briefly summarized in Table I.

Only four positive cultures were obtained in this series and all of them in the group of far advanced pulmonary tuberculosis where the patients were

*Löwenstein's Congo red egg medium.

TABLE I

RESULTS OBTAINED BY DIRECT CULTURE OF WHOLE BLOOD AND BY LÖWENSTEIN'S METHOD IN DETECTING TUBERCLE BACILLI IN THE BLOOD STREAM

DIAGNOSIS	NO. OF CASES	AGE	NO. OF CASES	NO. OF SPECIMENS	POSITIVE FOR TUBERCLE BACILLI	
					DIRECT CULTURE WHOLE BLOOD	LÖWENSTEIN'S METHOD
Advanced pulmonary tuberculosis (weak or critical)	46	20 to 63 yr.	28	36	1 (Mac.)†	0
		6 to 18 yr.	11*	14	0	1 (Mic.)‡
		9 mo. to 4 yr.	7	22	2§ (Mac.)	2§ (Mac.)
Pulmonary and bone tuberculosis (critically ill)	10	22 to 40 yr.	8	11	0	0
		17 yr.	1	3	0	0
		1½ yr.	1	1	0	0
Bone tuberculosis (pre- and post-operative)	6	22 to 36 yr.	6	9	0	0
Mesentery, cervical and intestinal tuberculosis	1	2 yr.	1	2	0	0
Actinomycosis of the jaw	1	3 yr.	1	2	0	0
Total			64	100	3	3

Total number of cases positive by both methods = 4

*In this group there were isolated two cultures of nonpathogenic acid-fast bacilli. In Case L14 a chromogenic saphrophyte was obtained in the portion of blood that was treated by Löwenstein's method, and in Case L 55 a single colony of a chromogenic acid-fast bacillus was found on the portion that was cultured directly on the Bordet-Gengou medium.

†Macroscopic growth.

‡Microscopic growth (see text).

§Both patients died after the last specimens were taken (See Table II).

either weak or critically ill. In two instances the cultures were positive by both methods; in the third, the "whole blood" culture was positive and the "Löwenstein" culture negative; and in the fourth, the "whole blood" was negative and the "Löwenstein" culture gave a doubtful positive (see Table II). In one of the cases in this series blood was obtained two and one-half hours before delivery and again five minutes after expulsion of the placenta in order to determine whether there was any blood invasion during labor. The blood of this patient remained consistently negative by both methods although a total of 5 specimens of blood were examined on four successive days. In one case of bone tuberculosis, blood was taken one hour before an operation on the spine and again one-half hour after the operation; both cultures gave negative results.

In two instances chromogenic, saprophytic acid-fast bacilli were isolated from the blood cultures. In Case L 14 the saprophyte was obtained on the portion of the specimen that was treated by the "Löwenstein" method and in Case L 55 on the "whole blood" culture. Both of these saprophytes were slow to appear on the original plates, but on subculture they grew freely both at incubator and at room temperature. This shows the need for careful identification of any cultures that are positive on smear, especially those that are atypical on colony morphology.

Table II shows the comparative results obtained by the whole blood culture and by the Löwenstein method in the four positive cases.

TABLE II

A DETAILED STUDY OF THE FOUR POSITIVE CASES SHOWING THE COMPARATIVE RESULTS OBTAINED ON DIRECT CULTURE OF WHOLE BLOOD AND AFTER TREATMENT BY THE LÖWENSTEIN METHOD

CASE	AGE	SPECIMEN	DATE	CONDITION OF PATIENT	RESULTS OF BLOOD CULTURE				
					DIRECT PLATING—WHOLE BLOOD			LÖWENSTEIN'S METHOD	
					AMT. CULT. IN C.C.	BORDET-GENGOU	CONGO RED EGG*	AMT. CULT. IN C.C.	CONGO RED EGG*
L 11	9 mo.	1st	2/ 1/34	Pulmonary and miliary T.B.	3.5	+ 1 col.	0	3.5	0
		2nd	2/ 8/34	Patient died 2/13/34	2.5	0	0	2.5	+
L 13	9 to 11 mo.	1st	2/ 1/34	Miliary T.B.	3.5	0	0	3.5	0
		2nd	2/ 8/34		2.5	0	+ 1 col.	2.5	0
		3rd	2/15/34		2.5	0	0	2.5	0
		4th	2/22/34		4.0	0	0	4.0	+
		5th	3/ 1/34		3.5	0	0	3.5	0
		6th	3/ 8/34	Patient died 3/15/34	4.0	0	0	4.0	0
L 20	46 yr.	1st	2/ 8/34	Pulmonary T.B. critically ill	3.0	+ 2 cols.	+ 1 col.	3.0	0
L 36	13 yr.	1st	3/ 1/34	Pulmonary T.B. weak	4.0	0	0	4.0	+ mic.†
		2nd	3/29/34		5.5	0	0	5.5	0

*Löwenstein's Congo red egg medium.

†Mic. = microscopic. No macroscopic growth was obtained after two months' incubation, but a smear of the scrapings of the culture showed a small clump of acid-fast bacilli. Subcultures of the scrapings of this culture and guinea pig inoculation gave negative results.

The results in this table show that the number of viable tubercle bacilli in the blood is extremely small and that they appear only intermittently even shortly before death. Two of the patients, L 11 and L 13, died soon after the last specimens were taken and both showed miliary tuberculosis. Yet in L 11 only one colony was obtained in the first specimen when 7 c.c. of blood was cultured and only a scant growth in 5 c.c. of the second specimen which was taken five days before death occurred. In Case L 13 only 2 of the 6 specimens gave a positive result: 1 colony from 5 c.c. of blood and a scant growth from 8 c.c. of blood. Case L 20 gave 3 colonies in 6 c.c. of blood. The first specimen of Case L 36 gave a doubtful positive culture on the portion that was treated by the Löwenstein method, although a total of 8 c.c. of blood was cultured. This culture remained macroscopically negative after two months' incubation, but a smear of the scrapings from the surface of the tubes showed some small clumps of acid-fast bacilli. Cultures and guinea pigs that were inoculated with these scrapings gave negative results. It seems likely that those were clumps of dead bacilli, otherwise they would in all probability have grown on subculture or guinea pig inoculation. A second specimen of 11 c.c. of blood gave negative results.

When the "whole blood" cultures are compared with the cultures by the Löwenstein method, we find that the results were better on the "whole blood" than on the "Löwenstein" cultures. There were 3 macroscopically positive cultures on the whole blood and only 2 on the Löwenstein culture. The culture that showed a few acid-fast bacilli in the scrapings of the Löwenstein tubes may be considered only as a doubtful positive.

There were few contaminations on the "whole blood" cultures. Although there were some contaminating colonies on the plates, none of the specimens were overgrown; whereas 7 of the "Löwenstein" specimens were overgrown by contaminations.

SUMMARY

A comparative study was made on 98 specimens of blood from 63 tuberculous patients and 2 specimens from a case of actinomycosis of the jaw, in order to determine whether the hemoglobin in human blood interferes with the growth of tubercle bacilli as stated by Löwenstein. The specimens were divided into 2 equal parts; one part of the blood was cultured on Bordet-Gengou and on Congo red egg mediums without any treatment, and the other part was treated according to Löwenstein's method.

It was found that the whole blood gave better results than the portion treated by the Löwenstein method; as there were 3 macroscopically positive cultures on the whole blood and only 2 on the Löwenstein cultures. In one additional Löwenstein culture a few small clumps of acid-fast bacilli were found microscopically when the surfaces of the tubes were scraped but subcultures and guinea pigs that were inoculated with these scrapings gave negative results.

Tubercle bacilli were found in only 4 of the 46 cases of advanced pulmonary tuberculosis, a large number of whom were critically ill, and 2 of the 4 patients that gave positive blood cultures died soon after the last cultures were made. All the other cases in this series gave negative results.

In two instances saprophytic acid-fast bacilli were found in the cultures. This shows the need for identifying carefully the cultures obtained, especially if they are atypical on colony morphology.

CONCLUSION

1. From the results obtained in this series it appears that the hemoglobin in human blood is not inhibitory to the growth of tubercle bacilli, since cultures of whole citrated blood gave better results than blood that was treated by Löwenstein's method to remove the hemoglobin.

2. Culturing whole blood has the particular advantage over Löwenstein's method of culture in that it requires no preliminary treatment and is, therefore, very much simpler and less time consuming. The use of plates instead of tubes as recommended by Löwenstein has the advantage of a large surface on which the specimen is well distributed and a slight contamination seldom overgrows the culture. We found in another investigation that when the number of tubercle bacilli is very small, growth usually takes place on the plates rather than on the tubes. Whether this is due to a better distribution of the inoculum, to a greater supply of oxygen or to other conditions, we have not been able to determine.

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STUDIES ON INFECTIONS OF HUMAN PARASITIC WORMS UNDER INSTITUTIONAL CONDITIONS*

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I. INTRODUCTION

MANY surveys have been carried out among population groups to determine the incidence of human entozoa. These surveys have included such population groups as armies, inmates of jails, hospitals, schools, orphans' homes, and asylums. The results of these surveys often give an indication of the incidence of helminth infections in a given geographic area that could not otherwise be easily obtained. This is particularly true when people are examined as they enter these institutions, the examinations of persons as they entered hospitals and jails having furnished valuable data concerning the incidence and intensity of infection and the geographic distribution of various species of human parasites.

During the past year the author carried out a survey at the Kankakee State Hospital (Illinois), adding similar data from another institution and locality.

II. MATERIALS AND METHODS

Arrangements were made for carrying out a survey at the Kankakee State Hospital to determine the incidence of helminth infection among the patients. The population of this institution is approximately 4,000 persons, including both males and females.

In this survey three different types of diagnostic methods were used: the direct smear, the Willis flotation method, and the method of perianal scrapings. In some instances all three types of diagnostic methods were used on the same patient, others received examination by two of the methods, while a third group was examined by only one method.

The direct smear was made in the usual manner, with the exception that decinormal sodium hydroxide was used as a diluent. The sodium hydroxide dissolves the mucus and admits a more even film preparation, at the same time freeing the ova from fecal material to a certain degree.

The Willis flotation method (Willis, 1921) with slight modifications was used to make a large number of the examinations.

Perianal scrapings were taken from the patients in the ward by a physician assigned for that purpose. They were taken by means of a blunt instrument, a considerable area of the anal folds and perianal region being scraped. The

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material was transferred to a slide on which a drop of decinormal sodium hydroxide had been placed. The drop of material was confined by a rectangular area which had been previously marked off on the slide by means of a wax pencil. The material was spread evenly over the area with a toothpick and immediately examined for the presence of ova, or was set aside for examination at a more convenient time. It was found that even though the preparation dried up, the ova (*Enterobius vermicularis*) were apparently not harmed enough to prevent an accurate diagnosis at a later time. When the examination was made at a later time a drop of water was added to the preparation and spread over the area so that visibility of the ova would be increased. These slides may be examined after several days or weeks and the ova will be only slightly deteriorated.

III. PROCEDURE AND RESULTS OF THE SURVEY

The survey was carried out during the winter months of the year 1932-33. Stools were collected and examined from patients on ten different wards. Data from one other ward were obtained when perianal scrapings were made. Table I gives the information concerning the number of examinations made by these various methods and the results obtained.

A total of 876 examinations were made on stools or perianal scrapings from 652 patients. Of these 115 were males and 537 females. Infection with

TABLE I

COMPARISON OF DIAGNOSTIC METHODS FOR ENTEROBIUS VERMICULARIS

WARD	NO. OF PERSONS ON WARD	EXAMINATION BY DIRECT SMEAR			EXAMINATION BY SALT FLOTATION (WILLIS-MOLLEY TECHNIC)			EXAMINATION BY PERIANAL SCRAPING		
		NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE
A-2-N	50				12	1	8.33			
A-3-N	47				13	0	0.00			
B-1-N	45				17	0	0.00			
B-2-N	44				22	0	0.00			
B-3-N	32				29	0	0.00			
1-N	206	89	0	0.00	194	12	6.19	128	45	35.16
3-N	25				19	0	0.00			
4-N	48				45	0	0.00	39	0	0.00
7-N	108				102	3	2.94			
8-N	146				52	1	1.92			
3-S	123							115	17	14.78
Total		89	0	0.00	505	17	3.37	282	62	21.99

only one species of helminth, *Enterobius vermicularis*, was noted. Seventeen or 14.8 per cent of the male patients examined were found to be infected with *Enterobius vermicularis*, while 57 or 10.6 per cent of the women were found to harbor this parasite. It must be taken into consideration when making comparisons that all men were examined by the perianal scrapings method only, while some of the women received examination by three different methods. Of all patients examined by various methods, 74 or 11.35 per cent were found

to be infected with this helminth. An adult worm was found in the stool from one of the female patients. Examinations were begun on the patients of ward 1-North and the first 89 stools collected were examined by both the direct smear method and the Willis flotation method. Examinations were continued on this ward and various women's wards by means of the Willis flotation method until stools from 505 patients had been examined. There were 3.37 per cent of these patients who were found to be infected with *Enterobius vermicularis*.

It was decided to continue examinations using a method which had been designed especially for diagnosis of infections of *E. vermicularis*, namely, the method of examination of perianal scrapings. Some of the patients were re-examined by this method and some new patients were examined. A total of 282 perianal scrapings were made and examined, including scrapings from 147 patients who had not been previously examined by another method. There were 21.99 per cent of the patients examined by this method who were found to be infected with *Enterobius vermicularis* as indicated by the presence of ova.

IV. EVALUATION OF TECHNIC USED

It is unusual that only one species of helminth has been found in a group of this type of persons. The results indicate that the methods employed failed to detect the ova, or that infections other than those detected were not present. An evaluation of the diagnostic methods used will be appropriate.

Perianal Scrapings.—The method of examination, although intended to be used primarily in the diagnosis of infections of *Enterobius vermicularis*, will detect the ova of other parasites if these infections exist. Cleinikow (1929) carried out a survey of Ufa using the perianal scrapings method. From 3,042 examinations, ova were found in the following percentages: *E. vermicularis* 89, ascaris 5.5, taenia 3, trichuris 0.8, *H. nana* 0.7, *Dicrocoelium lanceatum* 0.3, and *Diphyllobothrium latum* 0.03. These results show that ova of other helminths than *E. vermicularis* may be detected in the perianal scrapings.

The Direct Smear.—This method is considered reliable if sufficient numbers of smears are made from the same fecal sample, as high as ten smears being examined by some workers. In the author's experience light infections may not be detected if only one smear is examined, but there should be no difficulty in diagnosing the heavier infections. Davis (1924) in his experiments with egg-counting technics and methods of diagnosis comes to the conclusion that for practical purposes, the examination of one or two smears and the Willis technic give sufficiently reliable data for routine inspections. Freinkman (1930) states that the method of the plain smear, though simple, does not give very exact results and that methods of concentration are preferable. In comparative experiments he found that Fülleborn's method and Telemann's method (concentration methods) gave 75 per cent and 52 per cent positive results as compared to 8 per cent by the plain smear.

Willis Flotation Method.—Brine flotation methods will not detect infections of helminths that lay an operculate egg, due to the fact that the ovum ruptures at the operculum and sinks to the bottom of the vial. However, the ova of certain other helminths such as hookworm, trichocephalus, ascaris, *H. nana*

and taenia should be detected by this method. During the time that this survey was being carried out, the Willis flotation method was tested by means of a positive case of *Hymenolepis nana* from a source outside the institution. There was no difficulty in finding the ova in the film, even though the case was a light one.

From these discussions and the results of the author's test experiments, it would appear that other infections than *E. vermicularis* would have been detected if present, unless extremely light. It must be pointed out that these three methods are not equally efficient in detecting the presence of infections of *Enterobius vermicularis*. Table I gives us the range of this variability of incidence when different methods are used, ranging (in ward 1-North) from no infections in 89 examinations by the direct smear method to 35.16 per cent infection in 128 examinations of perianal scrapings, while the Willis flotation method would indicate that 6.19 per cent of 194 patients are infected with *E. vermicularis*.

A review of the literature of 28 surveys that were carried out on five different continents and a number of islands shows that there is a marked variability in the incidence of *Enterobius vermicularis*, as recorded. Part of this variability is no doubt due to a number of factors such as the type of population group surveyed, the age, and the environmental conditions. There is considerable evidence that much of this variability is due to the type of diagnostic method used.

Serbinow and Schulmann (1927) call attention to the fact that the method of perianal scrapings gives more accurate diagnosis of *Enterobius vermicularis* than any of the other methods that have been used. By this method they found that as high as 87.5 per cent of certain groups of children under institutional conditions were infected with *E. vermicularis*. They showed that the total percentage of incidence is increased if more than one examination is made.

Schauchat (1931) compared technic for the diagnosis of *E. vermicularis* using Fülleborn's method, perianal scrapings, and the search for worms in the stools after an anthelmintic was administered. Thus 299 cases were examined by the Fülleborn method and 10.7 of these cases were positive; 299 perianal scrapings were examined, and 48.2 per cent were positive. After giving an anthelmintic to 196 persons, the stools were searched and *E. vermicularis* was found in 72.9 per cent.

The results of the present survey support the evidence given by Serbinow and Schulmann (1927) and Schauchat (1931), that the perianal scraping is the only method that can be used in the diagnosis of infections of *Enterobius vermicularis* with any degree of reliability.

V. GENERAL CONSIDERATIONS

1. *Institutional Environment and Helminth Infections.*—As has been mentioned, it is strikingly unusual that infection by only one species of helminth was noted in a group of this type of persons. So far as the author is aware, these results have not been paralleled in any other survey of population groups. Two general reasons may be set forth. One is that the technic would

probably fail to detect certain infections, but it will detect certain other infections which one might expect in an institution or similar population group. Let us then for the moment give our consideration to a second factor, namely, the effect the institutional environment has on the presence of helminth infections.

The conditions and regulations of this institution are such that there seems to be little opportunity for the spread among the patients of infections that depend upon gross pollution of surroundings to effect this transfer. The buildings are of first-class stone construction, with hardwood floors and modern plumbing and heating fixtures. Bathrooms and washrooms are supplied with water from a central water supply coming from deep wells. There are self-flushing toilets in the washrooms. Equipment, interior of buildings, and dormitories are kept scrupulously clean.

The patients are not turned out into fenced inclosures as is the case in some institutions, and there is no chance for soil pollution. Untidy or careless patients are kept in a screened or enclosed porch, depending on the season of the year. Any sputum or excreta that may reach the floor are immediately removed and the floor scrubbed with disinfectant. There is no problem of soil pollution and the ova of these forms that require an incubation period outside of the host or must develop into a larval stage would no doubt be destroyed before reinfection could occur.

Lane (1913) in reporting on the incidence of infections in prisons and jails of Bengal, regards the possibility of infection in a jail as nonexistent, and expressed an opinion that the whole question of the prevention of infection is a matter of simple hygiene such as is obligatory in a jail. He states that under such circumstances a natural cure will ensue, provided the sentences are long enough.

Keller (1931) adds some interesting data of the same import while making a study of the parasites of children in one of the state institutions just outside Nashville, Tennessee. Under proper hygienic conditions, the group of patients who did not receive treatment over a period of fifteen months showed a reduction in incidence of 100 per cent in the cases of ascaris, 83.4 per cent in the cases of *Hymenolepis nana,* 75.6 per cent in the case of trichuris, and 59 per cent in the cases of hookworm infection. The Stoll dilution egg-count method was used in these studies. Chandler (1929) in studying the rate of loss and acquisition of hookworms stated that the degree of infection in an Indian jail had lessened by 92 per cent in five years but that the number of infected persons had not.

In making a study of the helminth infections in some of the institutions of Alabama for the insane and mentally defective, Caldwell, Caldwell and Davis (1930) found that there was an increase of infection with ascaris and trichuris in incidence and degree, and that infections with hookworms increased unless modified by treatment. They, however, found that this increase was brought about by dooryard pollution and continual contact with polluted soil. The patients were permitted access to walled enclosures, and soil pollution occurred within these areas.

Because of the unquestionable sanitary conditions prevailing at the institution under study, and because of the possibility of losing infection with the lapse of time when the individual is not being reinfected, one would not expect to find infections with those more common worms unless in the case of late admissions.

No information is available at this institution concerning the possible increase or decrease of *E. vermicularis*, since this is the first study made. It seems probable to the author that there is a possibility of spreading the infection because of the close contact that these patients have with each other, and because of the possibility of coming in contact with clothing that is contaminated with ova.

2. *Variation of Infection with E. Vermicularis on the Different Wards.*—By observing the table containing the results of these examinations one will notice that wards 1-North and 3-South have the highest percentage of infected patients. As the patients are admitted to the hospital they are taken to the receiving wards and left there until they can be classified. Personal habits, behavior, and physical condition are criteria used in classifying patients. There is considerable shifting of patients as their condition changes. The patients on wards 1-North and 3-South are classed as untidy. Mentally they are very much deteriorated, and they are very untidy in personal habits. Although the patients on ward 4-North were examined by both the Willis technic and by perianal scrapings, no infections were found. The author was impressed by the general good appearance of the patients on this ward and could note an interest in personal hygiene. The majority of the patients on this ward were classified as having some form of epilepsy or epileptic psychosis. This seems to bear out what would be expected, that the mental condition as it affects their personal habits influences the incidence of infection.

3. *Relation of Mental State to Parasitic Infection.*—When the patients are grouped according to the type of psychosis and compared with the incidence of infection, there seems to be little indication of a correlation. The data will bear a more thorough study from this standpoint. Marchand (1923) expressed an opinion that there might be a relation between convulsions due to helminth infections and epilepsy. However, in this survey no infections were found among the epileptic patients on ward 4-North. As stated above, it would appear that infections are influenced by the mental state of the persons and their habits as brought about or conditioned by the type of psychosis.

4. *Correlation of Age and the Incidence of Infection.*—In Table II information is given concerning the incidence of infection in relation to age. When considering the wards separately, with the exception of one or two, the number of patients in each age group is so small that a comparison cannot be made with any degree of accuracy. Considering all of the patients as a group there is no marked correlation of infection with the various age groups. The incidence in the age groups from twenty to twenty-nine and from thirty to thirty-nine seems to be uniformly lower than the age groups above them, but not markedly so. There were only three persons under twenty years of age and none of these were infected.

TABLE II

CORRELATION OF AGE AND INCIDENCE OF INFECTIONS

AGE GROUP	TOTAL INDIVIDUALS											INFECTED INDIVIDUALS (*E. vermicularis*)											PER CENT INFECTED											TOTALS		
	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	PERSONS	PER ONS INFECTED	PER CENT INFECTED
15-19						2					1						0					0							0						3	0	0.00
20-29		2	2	3	5	20	2	5	15	4	29		0	0	0	0	3	0	0	0	0	5		0	0	0	0	15.0	0	0	0	0	17.2	87	8	9.19
30-39	7	8	6	8	9	62	7	11	37	9	27	0	0	0	0	0	13	0	0	2	0	2	0	0	0	0	0	20.9	0	0	5.4	0	7.4	191	17	8.90
40-49	3	3	4	3	7	63	5	16	37	18	29	1	0	0	0	0	18	0	0	1	0	5	33.3	0	0	0	0	29.0	0	0	2.7	0	17.2	188	25	13.29
50-59	2		3	7	5	46	2	8	11	15	16	0		0	0	0	11	0	0	0	1	3	0		0	0	0	23.9	0	0	0	6.7	18.8	115	15	13.04
60-			2		3	30	3	8	2	6	13			0		0	7	0	0	0	0	2			0		0	23.3	0	0	0	0	33.3	67	9	13.43
Age not stated				1											0											0								1	0	0.00

VI. SUMMARY

1. A survey was carried out at the Kankakee State Hospital to determine the incidence of helminth infections among the patients.

2. Methods of diagnosis used were the direct smear, the Willis flotation method, and the perianal scrapings method. Some patients were examined by all three methods. Slide preparations of perianal scrapings set aside for several hours or days before diagnosis gave accurate diagnosis of *Enterobius vermicularis*.

3. Of 652 patients 876 examinations were made. Only ova of *E. vermicularis* were found, being present in 74 or 11.35 per cent of all of the patients examined. Some wards were negative: in other groups 35 per cent of the individuals examined were found infected with this helminth.

4. An evaluation is given of the diagnostic methods used. Results indicate that the examination of perianal scrapings is the only method that approaches reliability in the diagnosis of *Enterobius vermicularis* infection. When using this method, examinations should be made at several different times before a final negative diagnosis is given.

5. The institutional environment is such that little opportunity is afforded for the spread of this infection among patients if it depends on gross pollution of surroundings. Enterobius may possibly be spread from person to person through contact with soiled clothing, or because of intimate association. Other infections that may be present when the patient is admitted, such as ascaris and trichocephalus, will likely be lost without further spread.

6. Infection with *E. vermicularis* varies in the different wards. The wards having a high percentage of mentally deteriorated individuals have a higher incidence of infection.

7. There is little correlation between the type of psychosis and the infection other than that the psychosis influences the habits of the individual, in some cases predisposing to helminth infection.

8. There is no marked variation in the incidence of infection with *E. vermicularis* among the different age groups.

The author wishes to express his appreciation to Dr. Henry B. Ward for his interest and encouragement in carrying out this survey and for the many helpful suggestions received. Thanks are also due Dr. G. W. Morrow, Acting Managing Officer of the Kankakee State Hospital (Illinois), for placing before me the facilities of the hospital which made it possible to carry out this survey.

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THE INFLUENCE OF CERTAIN LIPOIDS ON THE GROWTH OF A RABBIT NEOPLASM*

ALVIN R. HARNES, M.D., NEW YORK, N. Y.

EARLY in the study of a malignant tumor, isolated from the rabbit by Brown and Pearce,[1] two peculiarities were noted. First, metastasis of the tumor occurred rarely in the brain or central nervous system of the animal. Second, the tumor would rarely grow from a primary innoculation in the brain of the rabbit. Later studies by Harnes[2] showed that animals with a high whole blood cholesterol recovered from the tumor, while those animals with low whole blood cholesterol died. It was also pointed out in this study[2] that as the tumor progressed in growth and metastasis, the whole blood cholesterol determinations increased in value.

With these facts in mind it was thought desirable to study the effect of certain lipoid extracts of brain tissue on the growth and metastasis of this rabbit neoplasm.

MATERIALS AND METHODS

The cerebrum, cerebellum, and medulla of calves, aged from six to eight months, were obtained and the extract prepared within twenty-four hours after the animal was killed. After removing the meninges, the brain tissue was finely minced and placed in a liter flask. Three hundred cubic centimeters of neutral 95 per cent C_2H_5OH were then added and the whole refluxed under vacuum at 70° C. for one hour. The alcohol was then drained off and filtered. After removing the alcohol by distillation the residue was taken up with corn oil in sufficient amount to make a 50 per cent solution of brain extract. This solution will hereafter be spoken of as brain extract.

*From the Department of Pediatrics, Cornell University Medical College and the New York Hospital.

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The rabbits used in this experiment were of mixed breeds and from six to eight months of age. They were kept in the same room and given the same diet. The 33 animals were divided into three groups: 11 for controls, 10 for corn oil, and 12 for brain extract. Prior to the time of innoculation with the tumor, the latter group received 5 c.c. of brain extract subcutaneously on alternate days until a total of 30 c.c. had been given. The other received 2.5 c.c. corn oil in like manner on the same days. On April 15, 1933, all animals were innoculated in one testicle with 0.3 c.c. of a tumor emulsion. The animals described above continued to receive brain extract and corn oil until the termination of the experiment on June 12, 1933. On this date all animals were killed and autopsied.

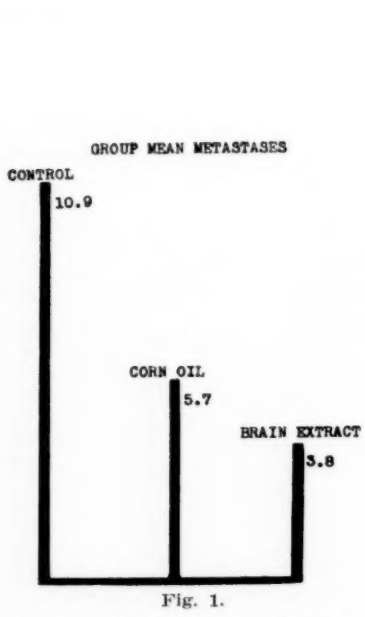

Fig. 1.

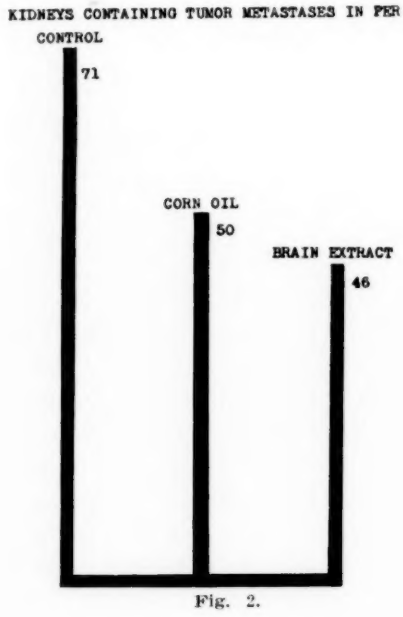

Fig. 2.

Fig. 1.—Group mean metastases.
Fig. 2.—Kidneys containing tumor metastases in percentage.

RESULTS

The results of the observations made in this experiment are presented in the form of tabulated summaries, Table I, and Figs. 1 and 2. The tabulation represents the number of metastases of the neoplasm per animal and includes the primary tumor.

DISCUSSION AND CONCLUSIONS

From the results presented in Table I it will be noted that the highest number of metastases occurred in the control rabbits. The mean value for the eleven animals in this group was 10.9 ± 0.71 with a standard deviation of 3.5. The number of kidneys free of tumor in this series was found to be 29 per cent (Fig. 2). The volume or amount of tumor was greatest in these animals.

Those animals receiving corn oil had the best appearance clinically. They maintained a constant weight and were entirely free of snuffles at the termina-

TABLE I

NUMBER OF METASTASES PER ANIMAL

11 CONTROLS	10 CORN OIL	12 BRAIN EXTRACT
8	14	2
15	8	4
12	2	8
16	2	1
12	5	7
12	6	2
9	5	4
4	6	6
15	6	5
8	3	3
9		1
		3
Mean $= 10.9 \pm 0.71$	Mean $= 5.7 \pm 0.73$	Mean $= 3.8 \pm 0.48$
St. D. $= 3.5$	St. D. $= 3.25$	St. D. $= 2.34$

tion of the experiment. The mean value for the ten animals in this series was 5.7 ± 0.73 (Fig. 1) with a standard deviation of 3.25. Fifty per cent of the kidneys were free of metastatic tumor.

The twelve animals receiving the brain extract had the least amount of tumor and number of metastases. Clinically these animals were in very poor condition, having lost considerable weight, and many were developing snuffles. The mean value for metastatic tumor in this group was 3.8 ± 0.48 with a standard deviation of 2.34. Fifty-four per cent of the kidneys were found free of neoplasm.

SUMMARY

The results of an experiment are presented in which it is shown that an alcoholic extract of brain and corn oil retard the growth and metastasis of a malignant rabbit neoplasm. The average number of metastases for the control animals was found to be 10.9 ± 0.71; for those animals receiving corn oil 5.7 ± 0.73; and those receiving brain extract 3.8 ± 0.48. The standard deviation for these groups was 3.5, 3.25, and 2.34, respectively.

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LABORATORY METHODS

AN EXPERIMENTAL BLOOD SAMPLING PROCEDURE*

GEORGE S. SAMUELSEN, B.S., BROOKLYN, N. Y.

METABOLIC studies involving blood chemistry procedures upon rabbits have been greatly hampered by the difficulty encountered in obtaining the necessary blood samples. It was an attempt to remedy this condition that resulted in the development of the following procedure. This method is a simplification of one in common use, but has the added advantage of being rapid, of being performed without the aid of an assistant, and apparently causing but slight damage to the animal.

Procedure.—The rabbit is allowed to sit unrestrained upon a table. The operator's hands are then placed one on each side of the animal (Fig. 1), in

Fig. 1.

such a manner that the location of the heart is clearly defined. Now let the index finger of the left hand indicate the point of maximum beat; this is usually the fourth interspace. A 23 gauge needle having a one-inch shaft, attached to a convenient size syringe, is introduced into the chest wall of the animal at the indicated point, in such fashion as to keep the needle and syringe always in a horizontal plane. The heart will usually make its location known by the intermittent beating against the needle point. Upon introducing the needle still further, the wall of the heart is pierced. A slight withdrawal of the plunger will cause blood to be drawn into the syringe (Fig. 2). In many instances it is unnecessary to withdraw the plunger for the pressure within the heart will cause an unrestrained plunger to be forced slowly out of the syringe

*Received for publication, December 31, 1934.

by the inflow of blood. When the desired amount of blood has been obtained, the needle is withdrawn in the same horizontal plane as introduced.

Comment.—In order to test the value of this procedure, about fifty rabbits were subjected to the treatment outlined above, as often as ten times in one day and fifty to sixty times within a six-week period. There was not the slightest sign of injury to even a single animal of the entire series. This, combined with the picture resulting from autopsy of many animals, shows this method to be practical and worthy of recording.

Fig. 2.

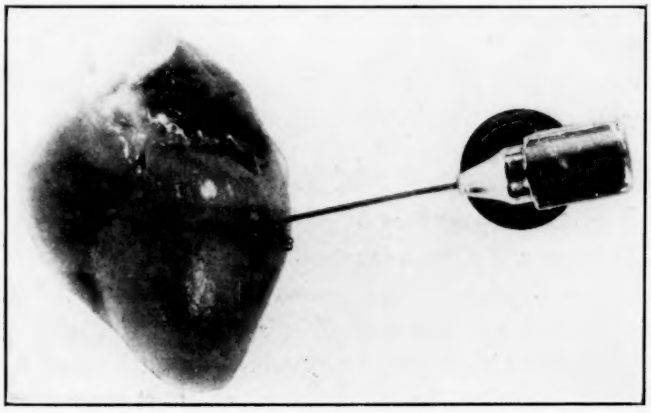

Fig. 3.

In many cases, the needle was introduced into the heart as described, and the animal killed in order fully to describe the point of penetration. In all cases there was no indication of hemorrhage. The points of introduction of the needle were completely closed. In all cases the needle pierced the left ventricle at a point on the base of the heart between the interventricular line (*A*, Fig. 3), and the circumflex branch of the left coronary artery (*B*, Fig. 3). Dissection of the heart showed that the needle entered the left ventricle just above the papillary muscle, but below the mitral valve.

The success of this procedure lies primarily in the following facts: (1) The rabbit being in the sitting position presupposes a normal and almost constant location of the heart. (2) The introduction of the needle from the left side of the animal ensures the needle striking the left ventricle. (3) Besides striking the heart at the point where the muscle wall is thickest the ventricle itself at this point has the greatest depth, thus allowing the maximum variation extent to which the needle may penetrate, without piercing the interventricular septum. This control of the extent to which the needle is introduced is further checked by using a needle of standard length.

The author takes this opportunity of expressing his sincere appreciation to Drs. Matthew Steel and G. B. L. Smith for their kind interest and suggestions.

ON A NEW MODEL OF PIPETTE FOR THE ERYTHROCYTE SEDIMENTATION TEST*

GILBERTO G. VILLELA, RIO DE JANEIRO, BRASIL, S. A.

IN THEIR research work Faraeus and numerous other investigators have shown the value of the relative erythrocyte sedimentation rate in connection with infectious diseases, especially in tuberculosis and leprosy. Since then many different types of instruments have been brought out for measuring the speed of sedimentation.

Linzenmeier adopted as a measure, the time which the erythrocyte column takes to sink as far as 18 mm. in a tube of special width. This technic did not prove practical, especially in cases where the sedimentation was very slow, thus causing considerable loss of time.

The pipette recommended by Westergren is more accurate, and with it readings can be taken every hour, thus making it possible to plot the curve of the drop of the blood corpuscle volume during the time of the procedure.

Westergren's first model consisted of a glass tube of even thickness, to which a ruler, scaled in millimeters, was fixed and through which the corpuscular column, inside, could be read. Later, more nearly perfect tubes for direct reading were made, having the graduations marked on the glass itself. The greatest drawback of these models was the difficulty in keeping the blood column from leaking through the lower end of the tube. The hematocrit tube is always accompanied with a wooden support or pedestal to which a metal plate is fixed, for the double purpose of keeping the tube in an upright position and preventing the blood from escaping. But even with very careful handling, it was difficult to ascertain the level the blood had reached, and at the same time prevent its leaking out at the lower end of the tube.

*From the Oswaldo Cruz Institut.
Received for publication, December 10, 1934.

Adler recently tried to correct these deficiencies by fitting a piece of rubber, shaped like the finger of a glove, on the upper part, which also acted to retain the blood, by suction.

The Adler pipette widened out at the upper end and was fastened to a rest or prop by means of two side hooks which compressed the rubber band, to stop the blood from escaping.

In this description, we are proposing a new and simplified model, which is easily handled and which appears to us to overcome, and do away with all the defects of former types.

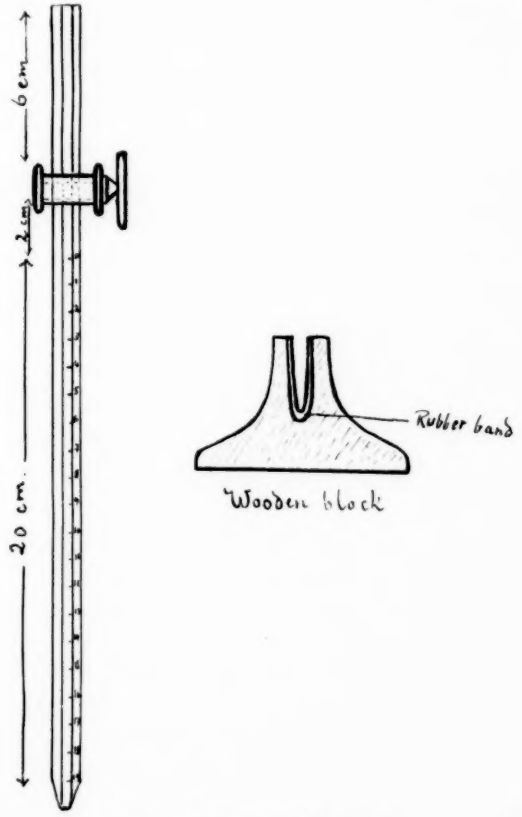

Fig. 1.—Sedimentation pipette.

Our sedimentation tester is composed of a tube similar to Westergren's, that is, it is the same in width and also scaled to millimeters, but it has a tap attached to the upper end, which allows perfect control over the levelling of the contents of the tube, so that both Westergren's pincers as well as Adler's fittings of rubber may be dispensed with (see Fig. 1). Besides this it may be noted how easily the pipette can be operated. It can be emptied at will and regulated by a mere turn of the tap.[1] The pipette is kept upright in a little wooden stand with a small hole in it, which adapts itself perfectly, with the aid of a circular rubber band on the edge of the rim in which the tube is placed.

[1]The tap must be lubricated with lanoline or other kind of grease to avoid the loss of air.

The readings can be taken exactly as with Westergren's apparatus, and the results may be compared because the diameter and the scaling of the tube are identical.

The technic for the determination is simple:

Place 0.1 c.c. of a 15 per cent solution of neutral potassium oxalate in a test tube, and then dry it carefully by holding it over a flame. Draw, by venipuncture, 5 c.c. of blood and introduce it immediately into the tube and shake it slightly. Draw blood up in the pipette to a little above the sign *O*, and turn the tap off. Fix the level with a little movement of the tap and wipe away the blood from the outside wall of the tip of the tube with a piece of filter paper. A concave mêniscus forming at the lower end of the pipette, will show that the shutting off by the tap was absolutely successful.

Now place the pipette upright on the opening of the wooden block and make readings at one, two, six and twenty-four hours from the time the blood was drawn.

A NEW DEVICE FOR OXYGEN ABSORPTION IN GAS ANALYSIS APPARATUS*

F. S. Cotton, D. Sc., Boston, Mass.

IT IS doubtless the experience of all workers in the field of gaseous metabolism that potassium pyrogallate solutions are frequently found to be unduly slow in absorbing oxygen, so necessitating the tedium of many trips to the absorption pipette.†

Further, the adoption of the Krogh type of absorption pipette where greater reliability of analysis is in question, accentuates still more this tedium. The Krogh pipette (series of pear-shaped guttered bulbs) is shown in Fig. 1, *A*. This pipette eliminates the risk of entangling small air bubbles by increasing liquid surface without resorting to the original series of hollow internal tubes, but in abandoning the latter, sacrifices an extensive amount of useful surface, and so lengthens (relatively) the absorption process.

This drawback to the Krogh improvement has stimulated the production of various devices to obviate the trouble. All of these involve at least sufficient complexity to increase distinctly the expense of manufacture, while the presence of moveable parts in some involves in addition the possibility of clogging with use, or of occasionally entangling a small gas bubble. Nothing but very extensive use could yield a pronouncement on the latter points. Shepherd, Martini, and Margaria have each described devices for the purpose in question.

The justification for yet another device is that it is simple of construction, works automatically, and having no moveable parts, cannot become clogged or entangle air bubbles.

*From the Fatigue Laboratory, Morgan Hall, Harvard University.
Received for publication, January 16, 1935.

†There is nothing in the literature to account for this irregular behavior, and research instituted along the lines of catalysis with the object of hastening the process has so far been fruitless.

The objects of the present design are:

1. To extend the stay in the pyrogallate of the last half of the air which visits it, which half is, of course, normally the first to return.

2. To promote the amount of contact between gas and fluid surfaces in the pipette.

3. To keep fresh pyrogallate flowing over the glass surfaces, which typically drain only too readily.*

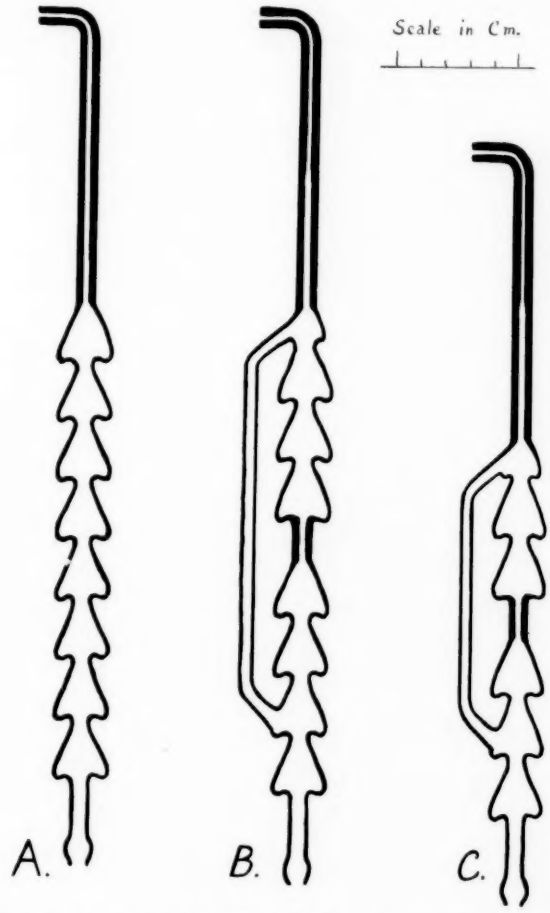

Fig. 1.—*A*, Krogh pipette. *B*, modified device for 20 c.c. gas machine. *C*, modified device for 10 c.c. gas machine.

The way in which these objects are attained may be illustrated by reference to Fig. 1.

The first object is secured as follows: As the gas is forced over into the pipette (Fig. 1, *B*), the pyrogallate is driven more quickly through the side tube than through the series of bulbs, since the constriction shown offers more resistance to flow. Approximately when the first half portion of gas has entered the pipette, it has begun to pass through the lower end of the side tube, so that most of the second half portion is thus manipulated below the first and, therefore, retreats after it when the gas is withdrawn from the pipette.

*Hence the gutters in the Krogh bulbs which are designed to restrict too radical a drainage. Further, the mechanical pump of the 40 c.c. Carpenter machine for chamber air analysis is a practical proof of the value of continuous irrigation.

The second object is secured in two ways: (a) When the second half portion of gas enters the main body of the pipette by the lower end of the side tube, the gas bubbles up through the fluid, so securing optimal physical contact. Moreover as soon as a cubic centimeter or so collects there, it is sprayed from above by the descending jet of pyrogallate traveling fast through constriction. (b) When the withdrawal of gas commences, the pyrogallate is very quickly drawn back up the side tube, and, spilling over into the gutter of the top bulb descends as a curtain of fluid all round as the air retreats, thus bringing fresh fluid into contact with this air.

It remains to quote precise facts about the relative efficiency of this device in comparison with the original Krogh system of bulbs.

This comparison was made by using the same solution of potassium pyrogallate to absorb the oxygen from samples of air in each of the pipettes in ques-

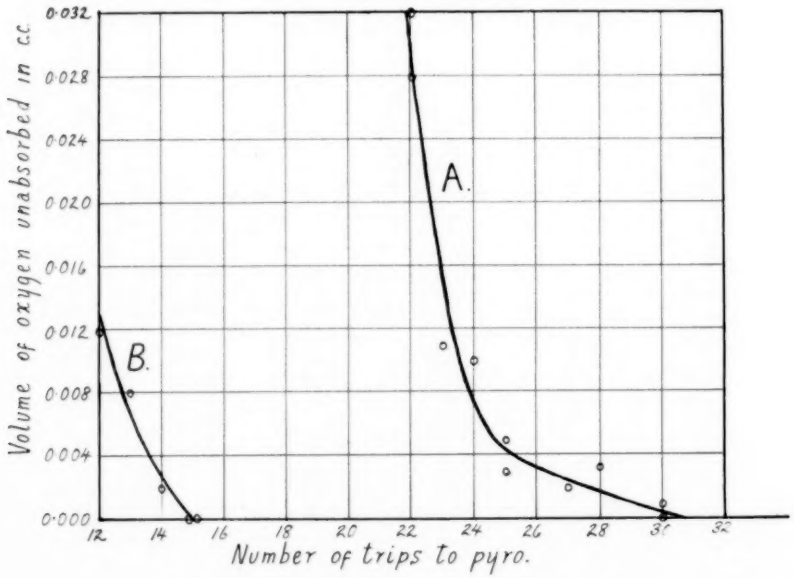

Fig. 2.—*A*, Krogh pipette. *B*, Present device.

tion. The number of trips to pyrogallate requisite to absorb completely the oxygen was ascertained in the following manner. In the case of each sample of air, of approximately the same initial volume, a certain number of trips (n_1) were made to the pyrogallate and the resultant volume observed. A further number of trips was then made in excess of the number required for complete absorption of the oxygen, and this resulting volume also was recorded. On repeating this procedure a number of times, sufficient data were obtained to plot the residual volume of oxygen (difference of the reduced readings on the buret) against the initial number of trips to pyrogallate (n_1).

Fig. 2 shows the result in the case of a Krogh pipette for absorbing oxygen from a 20 c.c. sample of air, and in the case of the present device.

The graph shows that whereas with the unmodified Krogh bulbs the necessary number of trips to pyrogallate was more than thirty, the number required

in the case of the present device was fifteen. Hence, this device cuts down the number of trips to approximately one-half. The same procedure was also adopted in comparing the Krogh pipette of a 10 c.c. Haldane apparatus with the device in question of appropriately reduced size. In this case the increased efficiency is not so great as when larger volumes are concerned, the number of trips to pyrogallate being reduced to three-quarters in comparison with the reduction to one-half in the first case.

The requisite diameters of side arm and constriction had, of course, to be determined by trial, so that the optimal result was obtained only after a number of experimental pipettes* had been constructed, in which these measurements were suitably varied.

The dimensions found suitable for the pipettes of the two sizes discussed in the text are given below, the diameter in each case being the internal diameter. The same dimensions apply to both pipettes which differ in the number of bulbs and position of the constriction.

Bulb dimensions—

Neck of bulb	diameter	4.0 to 5.0 mm.
Maximum width	diameter	19.0 to 20.0 mm.
Height of bulb		15.0 to 16.0 mm.
Side tube		2.9 to 3.1 mm.
Constriction	diameter	2.3 to 2.4 mm.
	length	14.0 to 16.0 mm.
Capillary tube above bulb—		
Leading from rest of apparatus	diameter	1.8 to 2.0 mm.
Short length above top bulb	diameter	2.6 to 2.8 mm.

The longer linear dimensions are deducible from the attached scale of 5 cm.

SUMMARY

A device is described for use in gas analysis machines to render more efficient the contact of gas and fluid in absorption pipettes, and so to reduce the number of trips necessary for complete absorption of the gas analyzed.

By this means the number of trips may be reduced to one-half or three-quarters that which is normally necessary for the type of pipette in current use for accurate quantitative absorption of oxygen by potassium pyrogallate, the extent of the saving being greater where larger volumes of gas are concerned.

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*My thanks are due to Arthur H. Thomas Company of Philadelphia for their generosity in supplying these experimental pipettes.

A GENERALLY APPLICABLE METHOD FOR THE ENUMERATION OF MICROSCOPIC OBJECTS*

A. C. Fay, Ph.D., Manhattan, Kan.

IN THE various fields of scientific investigation it is frequently desirable to enumerate microscopic objects such as bacteria, yeasts, mold spores, blood cells, intestinal parasites or their ova, protozoa, etc. Some of these forms may best be seen with the low power, others with the high dry or the oil immersion objectives of the microscope. Fay† has suggested a method using the oil immersion objective for the enumeration of bacteria, the general principles of which may easily be adapted for general use with the other objectives of the microscope. The method is as follows:

Place 0.1 ml., or 0.1 gm., of the specimen to be examined on a chemically clean 3 by 1 inch glass slide. Quantitatively diluted specimens may be used. If necessary to facilitate spreading, a few drops of water, stain or other liquid may be added to the slide; this need not be included in the calculation of quantitative dilution of the original specimen. After spreading the specimen evenly over the entire area of the slide, the preparation may be dried, fixed and stained by a suitable process or examined in a wet unstained condition, depending upon the nature of the material. The size and number of the objects to be counted will determine which objective of the microscope should be employed.

With the aid of a stage micrometer, adjust and record the tube length of the microscope which will give diameters of 1.57, 0.351, and 0.157 mm. for the fields of the low power, high dry, and oil immersion objectives, respectively. The areas of the respective fields will be 0.003, 0.00015, and 0.00003 square inches which represent 1/1,000, 1/20,000, and 1/100,000 of the area of the slide. Obviously if 0.1 ml. of the specimen is employed, the average number of objects counted per field should be multiplied by 10,000, 200,000, or 1,000,000 as indicated in Table I to give the count per milliliter. If more or less than 0.1 ml. of sample is used, suitable adjustment of the factor can be made. In order to insure a fair count, representative fields at widely separated parts of the slide should be selected at random.

In certain types of work it is more convenient to count the total number of objects in a continuous strip across the short dimension of the slide. In this case if the diameters of the fields for the three objectives are adjusted to 1.52, 0.381, and 0.152 mm., respectively, the areas of the corresponding strips represent 1/50, 1/200 and 1/500 of the entire area of the slide. As indicated in Table II, when 0.1 ml. of sample is employed, the total number of objects counted

*From the Kansas Agricultural Experiment Station.
Received for publication, January 31, 1935.
Contribution No. 161, Department of Bacteriology.
†Fay, A. C.: J. Dairy Sc. **16**: 311, 1933.

in one strip should be multiplied by 500, 2,000, or 5,000 to give the number per milliliter. This method obviates the necessity of keeping a record of the number of fields observed, but it should be employed only when the slide is moved by means of a mechanical stage. The diameters suggested in Tables I and II usually can be obtained with a 10X ocular.

It is frequently advantageous to insert into the eyepiece an ocular disc marked into quadrants and with one circle slightly smaller than, and concentric with, the microscopic field. If the ocular disc is used, the diameter of the concentric circle rather than of the whole field is adjusted as indicated in Tables

TABLE I

FACTORS FOR CONVERTING THE AVERAGE NUMBER OF OBJECTS PER FIELD INTO THE COUNT PER MILLILITER WHEN 0.1 ML. SAMPLE IS EMPLOYED

OBJECTIVE	DIAMETER OF FIELD IN MILLIMETERS	FACTOR
Low power (16 mm.)	1.570	10,000
High dry (4 mm.)	0.351	200,000
Oil immersion (1.9 mm.)	0.157	1,000,000

TABLE II

FACTORS FOR CONVERTING THE TOTAL NUMBER OF OBJECTS IN A STRIP ACROSS THE SHORT DIMENSION OF A SLIDE INTO THE COUNT PER MILLILITER WHEN 0.1 ML. SAMPLE IS EMPLOYED

OBJECTIVE	DIAMETER OF FIELD IN MILLIMETERS	FACTOR
Low power (16 mm.)	1.524	500
High dry (4 mm.)	0.381	2,000
Oil immersion (1.9 mm.)	0.152	5,000

I and II. A 6.4X ocular is most suitable for this purpose. The use of the ocular disc restricts the vision to the center of the field and eliminates from consideration the periphery where definition is frequently indistinct.

The method is being presented because it is believed that the fundamental principles involved and the values for standardization of the microscope may be useful in a number of fields of scientific work. Details of the procedure for the preparation of slides, the choice of volumetric or gravimetric samples, the extent of dilution, the staining, etc., which would make this method of enumeration directly applicable to the work of the bacteriologist, parasitologist, mycologist, pathologist, and others necessarily will be different for each specific use.

THE DETERMINATION OF LIPID PHOSPHORUS*

Alcohol Ether Soluble Phosphorus

Frances Krasnow, Ph.D., A. S. Rosen, D. Phar., and Y. Porosowska,
New York, N. Y.

LONG-CONTINUED use of the method of Krasnow and Rosen[1] for the determination of lipid phosphorus† has resulted in the introduction into the original process of certain modifications. These are embodied in the present communication.

It has been found advisable to discard the gas-heated oven, owing to the practical impossibility of maintaining a sufficiently constant temperature with the gas as supplied in certain localities. The condition was corrected by the use of an electric oven, in which it was found that a temperature range of approximately 80 to 90° gives very satisfactory results, provided that the acid fumes be allowed to escape at such a rate that a considerable proportion of them is always present. This is accomplished in a 15 by 16 by 12 inch oven especially constructed to withstand the acid fumes.‡ With this equipment, ten samples (but no more) may be oxidized at one time; the reaction is complete in sixteen to eighteen hours. The effect of the above change is illustrated by figures shown in Table I, showing the number of milligrams of lipid phosphorus and *lecithin*§ calculated from the phosphorus value recovered from 100 mg. of commercial lecithin and 100 c.c. of whole blood respectively.

Table I

SAMPLE USED	GAS OVEN		ELECTRIC OVEN	
	P	Lecithin	P	Lecithin
1 c.c. of commercial Lecithin solution (0.1% in alcohol)	2.27	56.7	2.84	71.0
	2.27	56.7	2.86	71.5
	2.24	56.0	2.82	70.5
0.5 c.c. of whole blood	9.50	238.0	10.48	262.0
	8.56	214.0	9.00	225.0
	8.00	200.0	10.28	257.0

Since some of the measurements in the original technic have been changed, the details are here supplied:

Blood (0.5 c.c.) is pipetted into 10 c.c. of an alcohol-ether mixture (3:1) contained in a 50 c.c. volumetric flask, preferably fitted with a glass stopper, and

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†"Lipid phosphorus" is used throughout this paper to indicate the alcohol ether soluble phosphorus. The amounts of other phosphorus compounds which might appear in the alcohol ether extracts of the fluids studied have been stated to be negligible.

‡The construction of this oven was made possible through the cooperation of the Electric Heat-Control Apparatus Company, Newark, New Jersey.

§Although commercial lecithin is not pure lecithin, it served as a good working material since we used it for comparative purposes only.

brought to boiling by immersion in a water-bath. Digestion is continued for about three minutes. After cooling, alcohol-ether is added to the 50 c.c. mark and the whole shaken vigorously. The filtrate (25 c.c.) is transferred quantitatively to a casserole of 150 c.c. capacity and evaporated to dryness over a water-bath. Then 10 c.c. of HNO_3 (sp. gr. 1.42) is added and the dish rotated in such a way that the acid loosens the dried extract from the sides. One-tenth cubic centimeter of H_2SO_4 (sp. gr. 1.84), 5 c.c. of $KClO_3$ (saturated solution) and 4 c.c. of a mixture of saturated KNO_3 and a saturated solution of $NaNO_3$ (1:1) are introduced. The casserole is placed in the oven at 80 or 90° C. and digestion continued until the mixture becomes white. A safe interval is eighteen hours. The white crystalline residue is dissolved in water by heating over a small free flame and transferred to a centrifuge tube (15 c.c.). Including at least two washings, the total volume should not exceed 10 c.c.

Following the Tisdall method[2] the phosphate is precipitated with 1 c.c. of the strychnine-molybdate reagent and agitated three times during fifteen minutes. After being centrifuged for three minutes at high speed, 1,600 r.p.m., the supernatant fluid is decanted and the precipitate washed twice with water, each time centrifuging at least one minute, 1.0 c.c. of NaOH (1 per cent) is added and stirred until solution is complete. This is diluted with water to about 5.0 c.c. and transferred to a 50 c.c. volumetric flask fitted with a glass stopper. The centrifuge tube is washed twice with water, using 5.0 c.c. each time, and the washings added. Ten c.c. of K_4FeCN_6 solution (20 per cent) and 5.0 c.c. of HCl (sp. gr. 1.18) are introduced. The mixture is allowed to stand for ten to twelve minutes and made up to volume with water. Readings are made immediately against a phosphate standard, prepared as directed by Tisdall. The formula for calculating the lipid phosphorus, P, in 100 c.c. of whole blood is: $P = 20/R \times 10$ where 20 is the reading of the standard and R is the reading of the unknown. To obtain the corresponding "lecithin" content, the value of P is multiplied by 25.

Tests have shown that the blood may be kept overnight in the ice chest and the procedure outlined above may be conveniently interrupted at any point except after the addition of the concentrated HCl.

One of our previous reports on lipid phosphorus contended that "test tube" oxidations should be discarded.[3-10] However, it was felt that with the technic of Zahnd and Clarke for manipulating such oxidations in the determination of sulphur,[11] reproducible results might now be obtained. As in the oven method 1 c.c. samples of a solution of commercial lecithin (0.1 per cent in alcohol), were used. The average recovery for four series of estimations were 2.64, 2.92, 2.68, 2.80 mg. P per 100 c.c., respectively, with a final average of 2.76 mg. which compares well with 2.84 mg. The steps in the procedure after oxidation were those adopted for the oven method previously described.

These data become more significant when we compare them with figures obtained on the same solution by other methods recently recommended for lipid phosphorus.[12, 13] The respective recoveries are 1.96 and 0.88 mg. P. However, when, in the latter technic, all the steps referring to extraction were omitted, 2.85 mg. was recovered.

The comparison is presented in Table II.

Some of these methods were then compared as to their efficiency in the determination of lipid phosphorus in whole blood. Here, too, the results may be best presented in tabular form (Table III).

TABLE II

MG. LIPID PHOSPHORUS RECOVERED BY EMPLOYING DIFFERENT METHODS

	BENEDICT-THEIS	BLOOR	ZAHND-CLARKE	KRASNOW-ROSEN	PREGL[14]
	2.07	0.86	2.64	2.84	2.90
	1.83	0.86	2.92	2.86	2.90
			2.67	2.82	
			2.81	2.90	
Average	1.95	0.86	2.76	2.86	2.90
Remarks	Determination from another laboratory yielded 1.55	Extraction steps omitted 2.60 3.11			5 c.c. was used in these determinations

Note: Each of the above figures is an average of a series of determinations. Since commercial lecithin is impure, it may be wise for comparison purposes to use the result obtained by the Pregl method as 100 per cent and refer all figures to that as a standard. The per cent yields of phosphorus for the several procedures are respectively 67.3, 29.7, 95.1, 95.5, and 98.3 per cent, taken in the order given in the table.

TABLE III

MG. LIPID P RECOVERED FROM BLOOD BY DIFFERENT METHODS

NUMBER OF BLOOD	BENEDICT-THEIS	BLOOR		KRASNOW-ROSEN
		USING BLOOR EQUIVALENT 1 MG. LECITHIN = 3 C.C. N/10 KIO_3	USING AUTHOR'S EQUIVALENT 1 MG. LECITHIN = 2.8 C.C. N/10 KIO_3	
364	184	236	253	267
365	190	205	220	242
366	201	211	225	252
367	176	220	232	270
368	230	266	278	315
369	173	212	227	267

Although the results obtained by Bloor's method check those obtained by the Krasnow-Rosen technic quite well, there must be some compensating mechanism involved. As was previously shown, when a solution of commercial lecithin was treated as though it were blood and carried through all the steps in the procedure, the recovery was only 22 mg. $\simeq$ 0.86 phosphorus, whereas when the extraction part of the method was omitted, the yield was 2.85 mg. P. It seems then, that other substances are extracted from the blood and when oxidized, they compensate for the loss of alcohol-ether soluble phosphorus.

From our study, thus far, it appears that the oven method is to be preferred for routine work. The results are more accurately reproducible. Although the Zahnd-Clarke technic also yielded similar average figures, the proce-

dure requires more attention, so that for a large number of determinations, the former saves much time, both during the oxidation and during the evaporation of the alcohol-ether extract preparatory to oxidation.

With slight modifications involving volume adjustments, our technic has proved applicable to analysis of lipid phosphorus in spinal fluid and saliva, and total phosphorus in casein. Analyses of amounts lower than 0.1 per cent have been duplicated with a good degree of accuracy. A glance at the data given in Table III will illustrate this.

TABLE III

DUPLICATE ANALYSES

SUBSTANCE	MG. PER CENT		AVERAGE
Casein	0.78	0.83	0.81
	0.76	0.76	0.76
Spinal fluid	0.024	0.023	0.024
	0.023	0.024	0.024
Saliva	0.125	0.130	0.128
	0.360	0.390	0.375

Furthermore, although several thousand analyses have been made during the last three years the oven used shows no effect of acid corrosion.

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VITAL STAINING OF MALARIAL PARASITES*

A Preliminary Note

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ALTHOUGH the method to be described undoubtedly is known to many, no reference to it appears in the American or English literature. Malarial parasites stain readily with the so-called vital stains. Boucher[1, 2] has called attention to the usefulness of neutral red and Janus green in the study of the cytology of these organisms. Akashi[3] has also used this method, but his article is not available for reference. For some ten years the senior author has used vital staining with brilliant cresyl blue for demonstration and study of the malarial parasites. While making reticulocyte counts on malarial blood, Vryonis noted that marked changes in the appearance of the parasites and in their distribution in reticulocytes occurred after the initiation of quinine therapy.

Other vital stains have been studied. Neutral red colors the cytoplasm an irregular pink, the chromatin orange red, but the contrast is poor, and the method offers little advantage over unstained wet preparations. With Janus green the parasites stain pale bluish green, the mitochondria a more intense green. With mixtures of these stains such as are used for the study of leucocytes the appearances described are superimposed. With dilute Wright's stain dried on slides and used as a supravital stain, the contrast is exceedingly poor. With all three of the above methods, staining is so faint that it is of little aid except to an observer adept in vital staining and in the study of malarial parasites. Brilliant cresyl blue has the advantages of intense staining with fair differentiation of cytoplasm and nuclear material and of relatively low toxicity for the parasites, though it is more toxic than neutral red. Any technic which gives good reticulocyte staining will give satisfactory results with malarial parasites. The one which has proved most satisfactory in our hands is the following: Using glass prepared for vital staining a small drop approximating 2 c.mm. of saturated solution of brilliant cresyl blue in sterile physiologic salt solution is placed on a slide, a slightly smaller drop of blood is taken on a coverslip and dropped directly on the stain. The preparation is ringed with soft petrolatum. Staining is almost instantaneous, and the parasites remain alive for upward of two hours on the warm stage. Cytoplasm stains irregularly, presenting a coarsely stippled appearance, pale blue with slightly darker areas; chromatin is dark blue; pigment is readily visible.

Marked differences are seen between *P. vivax* and *P. falciparum*. The merozoites of *P. vivax* appear as pale blue pyriform objects with a single dark

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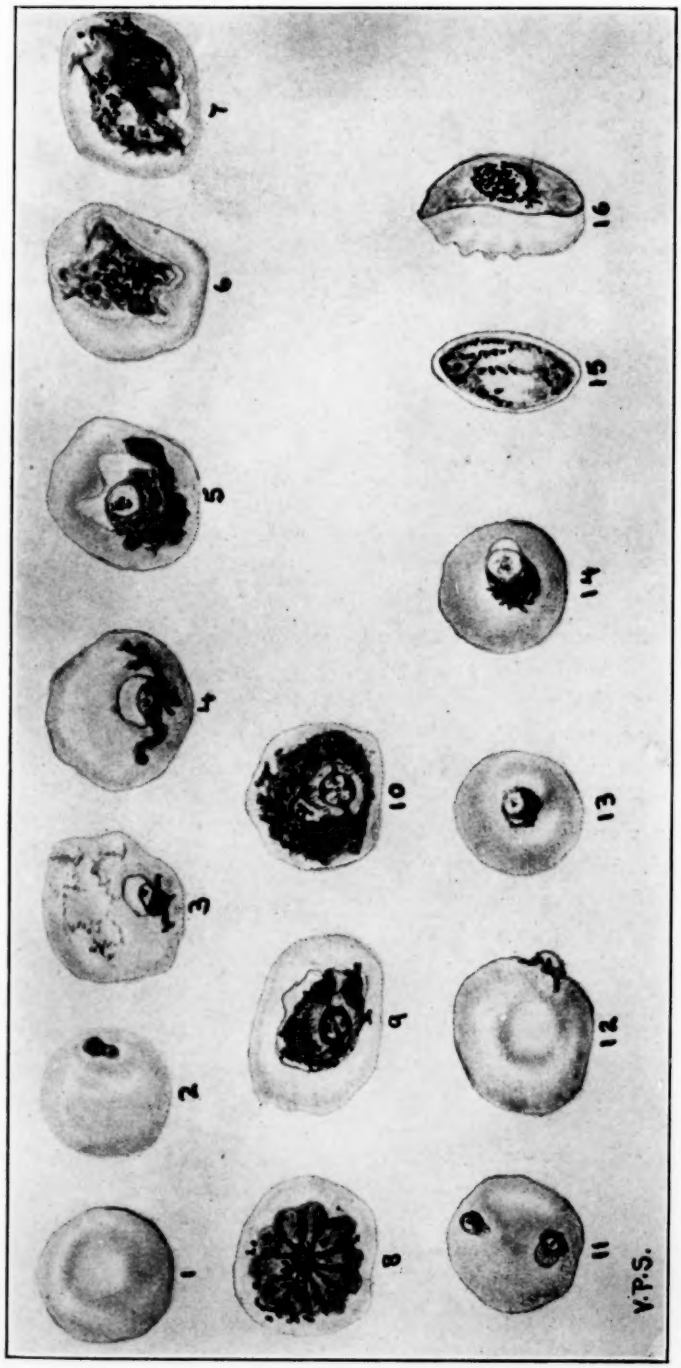

Fig. 1.—Sketched with the camera lucida at magnification approximately 2400. Somewhat reduced in photography. *1*, Normal erythrocyte; *2-10*, *P. vivax*; *2*, merozoite on surface of erythrocyte; *3*, ten- to twelve-hour "ring form" in reticulocyte; *4-8*, stages in development of schizonts; *9*, microgametocyte; *10*, macrogametocyte; *11-16*, *P. falciparum*; *11*, merozoites; *12*, "marginal" form; *13*, typical ring form; *14*, twenty-four-hour pigmented schizont; *15*, immature microgametocyte ("ovoid"); *16*, macrogametocyte.

blue granule in the smaller end. On attachment to an erythrocyte a pressure halo is visible for some time, and the parasite may be seen to move about on the surface of the cell. After entry to the red blood cell has been effected, an extraparasitic vacuole soon develops, and there is active ameboid movement. The extraparasitic vacuole persists through all stages of development. With age the dye is increasingly absorbed so that tertian parasites over twenty-four hours old are striking objects. Schizonts are easily recognized though no definite nuclear structure can be made out, gametocytes are readily identified, and in favorable optical planes the nucleus is readily seen. All stages of *P. falciparum* stain less intensely. Merozoites are smaller and more rounded, and on attachment to an erythrocyte they soon protrude a single blunt pseudopodium (Fig. 1, *11*). After entering a red cell, an extraparasitic vacuole is absent or exceedingly small, and ameboid movement is sluggish. So-called marginal forms show pseudopodia well embedded in the erythrocyte. Twenty-four-hour pigmented forms show many very small pseudopodia and occasionally a minute extraparasitic vacuole (Fig. 1, *14*). Mature schizonts have been observed but not under conditions when sketching was possible. The gametes stain characteristically (Fig. 1, *15*, *16*). Flagellation of microgametocytes has been observed in both species described. No opportunity has been afforded to study *P. malariae*. After death parasites of both species "round up" and show much the same morphology seen in stained dry smears.

After the initiation of quinine therapy in both species, ring forms have been found in nonreticulated erythrocytes only. The significance of this observation and its possible relation to Eaton's[4] hypotheses that the effect of quinine is on the parasites of greatest age and that the erythrocyte is susceptible to infection with malaria only during the reticulocyte stage, are subjects of present study. Under quinine the gametes of *P. falciparum* show striking changes in appearance, staining is more even and intense, the parasites are smaller, and their shape is distorted. Critical studies of the gametocytes of *P. vivax* under therapy have not been made.

The method offers certain advantages for demonstration and for the study of the malarial parasites. The organisms are well stained and easily recognized. They may be observed during life much more readily than in unstained fresh preparations. The morphology of the species under observation is easily differentiated by persons not trained in parasitology. The intracorpuscular location of parasites past the merozoite stage can definitely be demonstrated. It is likely that the method will facilitate study of the relation of parasites to reticulocytes and also of the mode of action of antimalarial drugs.

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PUNCTURE OF THE INTERNAL JUGULAR VEIN FOR DIAGNOSTIC PURPOSES*

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THE procedure of removing blood from the internal jugular vein is simple and apparently without danger. It would appear that while used in several clinics it might be more widely employed. While it is adaptable to patients of

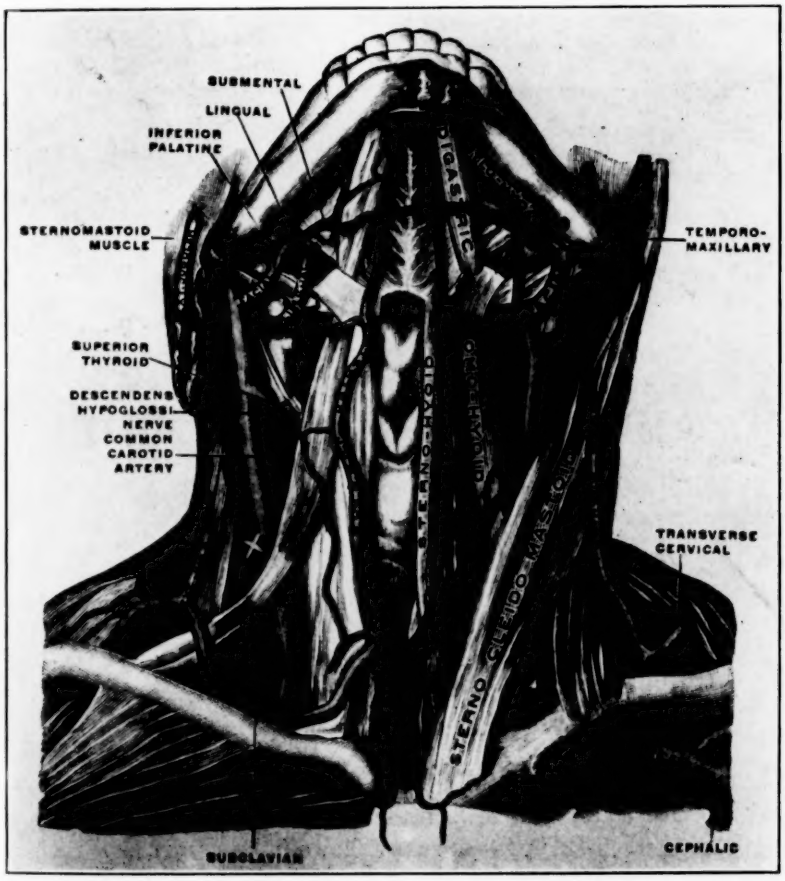

Fig. 1.—Illustration showing internal jugular vein on right, and *X* represents the point at which the needle enters the vein. (Spalteholz.)

all ages, it is particularly valuable in infants in whom, especially when they are dehydrated, it is difficult to find superficial veins which are readily accessible for the withdrawal of blood.

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TECHNIC

Either internal jugular vein may be used, the right being preferable since it is slightly larger and lies in a more favorable position. The patient is placed on his back with the head lowered over the side of the bed or the end of the table, so as to outline properly the sternocleidomastoid muscle and the external jugular vein. His head is turned as far as possible to the side opposite that on which the vein is to be punctured. The neck is painted with iodine which is subsequently removed with alcohol. The lobe of the ear should be similarly treated. A long needle with a small lumen, preferably a 20 gauge needle 2 inches in length, is attached to a syringe and, with the sternocleidomastoid muscle clearly outlined it is introduced just posterior to the point where the *external* jugular vein crosses this muscle at its posterior border. If the *external* jugular vein is not visible, the needle is then introduced at the junction of the upper and middle

Fig. 2.—Showing the starting point of the insertion of the needle under the sternocleidomastoid muscle Dotted line represents the direction which the needle takes under the muscle toward the sternal notch.

thirds of the posterior portion of the muscle. When introduced, the needle should be in a position almost parallel with the muscle and posterior to it but *inclined inward so as to point* toward the sternal notch. If the wall of the vein is not punctured when this procedure is followed, the needle may then be introduced almost its entire length, and, while withdrawing it slowly, gentle suction be made until the lumen of the syringe fills with blood.

The anatomical considerations of the internal jugular veins are demonstrated in the illustration. The glossopharyngeal and hypoglossal nerves pass between the artery and the vein; the vagus descends behind the artery and the vein and in the same sheath. At the root of the neck the internal jugular vein unites with the subclavian to form the innominate vein. The nerves are pushed aside if the needle is thrust in their direction, and no harm results. When the

needle is introduced almost parallel with the sternocleidomastoid muscle and kept just beneath it, no other structure will be interfered with. Myerson, Halloran and Hirsch[1] state that transfixing the walls of the jugular vein or of the internal carotid artery is not harmful, since they have repeatedly examined the vessels by open inspection after multiple punctures had been done.

CONTRAINDICATIONS

There are apparently no contraindications except infection in the neck or tumor masses in this region.

SUMMARY

It is recommended that puncture of the internal jugular vein be used more frequently for diagnostic purposes. In employing this procedure in over 1,000 instances and often in the same subject on several occasions no difficulty of any sort has been experienced.

[1] Myerson, Halloran, and Hirsch: Arch. Neurol & Psychiat. 17: 807, 1927.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

POLIOMYELITIS, Acute Anterior, Successful Vaccination Against, Kolmer, J. A., and Rule, A. M. Am. J. M. Sc. **188**: 510, 1934.

The vaccine used by Kolmer was prepared as follows:

1. Healthy adult Macacus rhesus monkeys, weighing 4 or more kg. of weight, were inoculated intracerebrally (frontal lobe) with 0.2 c.c. of a 5 per cent emulsion of monkey poliomyelitic cord (Rockefeller Institute strain) under ether anesthesia.

2. Symptoms of poliomyelitis usually developed in five to nine days and immediately after death, or when the infection was very severe, the hair was sprayed with 5 per cent tricresol solution and the skin over the head and back removed. The cords were then removed under aseptic precautions after the method of removing spinal cords from rabbits in the preparation of rabies vaccine by the original Pasteur method and 8 gm. of a mixture of several cords thoroughly emulsified aseptically in 100 c.c. of sterile physiologic saline solution.

3. After fine-mesh filtration under aseptic conditions there was added an equal volume of a sterile 2 per cent solution of sodium ricinoleate in saline solution.

4. This gave a 4 per cent suspension of mixed tissue in 1 per cent sodium ricinoleate.

5. The mixtures were well mixed and placed in an incubator at 37° C. for twenty-four hours and then in a refrigerator at 4° to 6° F. for fourteen days with daily shaking for a few minutes.

6. Subcultures were made and the vaccines kept in the refrigerator throughout the year. Even though sodium ricinoleate is of low bactericidal activity, the authors have found that the final concentration of 1 per cent is usually sufficient for sterilizing the vaccines when slight contamination had occurred in the removal of some of the cords and the preparation of the emulsions.

The authors have taken 0.5, 1.5 and 2 c.c. of the vaccine by subcutaneous injection every five days. The injections were accompanied by some stinging pain, especially after the first but less after the second and third injections, which quickly subsided, but there was little or no local reaction except in the 2 c.c. doses when the local reactions were comparable to those produced by the subcutaneous injection of rabies vaccine. Fever and constitutional reactions, however, were not produced, and, while they have found large doses of sodium ricinoleate by intramuscular injection capable of producing some tissue necrosis in rabbits, yet such were not produced in themselves by even 2 c.c. amounts of this poliomyelitis vaccine.

Fortunately for these experiments their blood sera before inoculation were free of antiviral properties, as determined by mixing 0.2 c.c. of serum with 0.2 c.c. of 5 per cent suspension of virus and injecting the mixture, after standing two hours, intracerebrally into two monkeys. Both animals developed poliomyelitis in about seven days.

That the 3 doses of vaccine produced antiviral antibody was shown, however, by repeating the test two weeks after the last dose of vaccine, when two additional monkeys inoculated intracerebrally with a mixture of 0.2 c.c. of serum and 0.2 c.c. of 5 per cent suspension of virus allowed to stand for two hours, remained perfectly well, whereas a control inoculated with virus alone developed paralysis in six days and finally succumbed. These results have shown, therefore, that the vaccine was apparently capable of producing antibody for the poliomyelitis virus and probably sufficient for engendering immunity.

It appears that this is the only effective method for determining whether or not a vaccine is capable of engendering immunity against poliomyelitis in human beings,

because an injection of virus as a test for immunity as done with monkeys is, of course, not permissible, and to vaccinate a group of children to determine over a period of years how many, if any, develop poliomyelitis as controlled by the incidence among a group of unvaccinated individuals would require the immunization of a very large number in the presence of an epidemic. The serum-antiviral test before and after vaccination, however, may be a quicker and more decisive test for acquired immunity, and especially when conducted with children, who are not as likely to have natural antiviral antibody in their sera.

Since these vaccines never produced the slightest evidence of infection in the monkeys or the authors, and believing that the amount of sodium ricinoleate employed effectively reduces the virulence of the virus, they are at present vaccinating a group of children varying in age from eight months to fifteen years. These have been selected after preliminary antiviral tests with monkeys have shown the absence of antibody in the sera. Three doses are being given at weekly intervals with monkey antiviral tests with the sera of some children after the first and second doses and with the sera of all children after the third dose in order to determine the rapidity of antibody production and the number and size of doses of vaccine required.

The first dose for children under three years has been 0.25 c.c. and 0.5 c.c. for older children up to fifteen. By making the first dose quite small in this manner and waiting seven days, opportunity is afforded for antibody production before the second and third doses are administered. Varying amounts are being given in the second and third doses and a subsequent report will soon be made on the results, along with further details on the preparation and standardization of the vaccine. Since the cord of a large-sized monkey will yield about 200 c.c. of vaccine, and taking a total of 3 to 4 c.c. for the immunization of a child, one monkey will yield sufficient vaccine for the immunization of 50 or more children. If subsequent experiments now under way show that it is possible to prepare vaccine of monkey poliomyelitic brain, the yield would be much larger and the cost correspondingly less.

DEXTROSE TOLERANCE, An Infection, Schmidt, E. G., Eastland, J. S., and Burns, J. H. Arch. Int. Med. **188**: 3, 466, 1934.

Seventy-three cases of infection have been subjected to the dextrose tolerance test. Among these were 36 cases of pyogenic infections, of which 18 were studied both during and after the infection, 9 of nonpyogenic febrile conditions and 28 of arthritis and rheumatism.

Among the 36 patients with pyogenic infection 29, or 72 per cent, gave distinctly pathoglycemic curves. The highest curves were obtained in cellulitis, the blood sugar on several occasions rising above 300 mg. and usually remaining elevated over three hours after the ingestion of dextrose. Despite such high blood sugar values, far above the generally accepted normal threshold of 180 mg., glycosuria was present in but 4 cases. When the infection had disappeared and the patient was ready to go home a second tolerance test almost invariably resulted in a normal blood sugar curve. Any previous tendency toward glycosuria had now disappeared.

Both streptococcic and staphylococcic organisms were effective in producing disturbances in the carbohydrate mechanism.

The 9 patients with nonpyogenic febrile conditions also yielded abnormally prolonged blood sugar curves.

Among 28 patients with arthritis and rheumatoid conditions 15, or 53.6 per cent, gave diabetic-like blood sugar curves.

The blood sugar values after fasting in pyogenic, nonpyogenic febrile and arthritic conditions were, with few exceptions, well within normal limits.

With 3 exceptions, cases in which obvious renal complications were present, the blood urea nitrogen level was within normal limits during the various infections.

PROTEIN, Serum, Simple Method of Estimation of, and Its Value in Tuberculosis, Pagel, W., and Stott, L. B. Tubercle, London **15**: 454, 1934.

Into six tubes is placed 0.5 c.c. of physiologic solution of sodium chloride. To Tube 1 is added 0.5 c.c. of fresh filtered ox bile, from which 0.5 c.c. of the mixture is transferred to Tube 2. This process is continued up to Tube 5, and 0.5 c.c. of the mixture from Tube 5 is discarded. The last tube[6] being a control tube contains only 0.5 c.c. of physiologic solution of sodium chloride. After this, 0.5 c.c. of 1:7 dilution of the serum to be examined is added to each tube and all tubes are heated until coagulation of the proteins appears in the last tube. The results are recorded immediately, and after twelve hours' standing at room temperature. There are all degrees of coagulation from intensive opacity like milk (++++), less opacity (+++), faint transparence (++), gradually falling (+±), to entire clearness (−).

SILICA, Inhaled, and its Effect on Normal and Tuberculous Lungs, Gardner, L. U. J. A. M. A. **103**: 743, 1934.

Dusts containing silica are preeminently dangerous. Present knowledge will not permit it to be said that only free silica is harmful; possibly some of the silicates will also be incriminated. One silicate, asbestos, produces a characteristic and dangerous type of pulmonary fibrosis. Silica is a tissue poison. In low dilutions it causes nodular fibrosis; in higher concentrations it produces rapid necrosis of cells of all kinds.

Human silicosis begins by damaging the pulmonary lymphatic apparatus and is followed by the development of nodular fibrosis of the parenchyma of the lungs.

Silicosis specifically predisposes to infection with the tubercle bacillus. The mechanism of this action has not yet been determined. It probably consists in some alteration in the soil rather than in changes induced in the infecting organism.

Nonsiliceous dusts localize about the lymphatic trunks and some of them excite the proliferation of small amounts of loose cellular connective tissue. They apparently do not increase susceptibility to tuberculosis.

Nonsiliceous dusts inhaled in combination with silica modify the action of the latter, altering the anatomic characteristics of the lesions and apparently decreasing the susceptibility to tuberculosis.

TUBERCULIN TEST, Quantitative, As an Index of Tuberculous Activity, Badger, T. L., and Myers, W. K. New England J. M. **211**: 241, 1934.

A total of 207 individuals have been tested with measured dilutions of tuberculin to determine what relation, if any, exists between the reaction to such measured dilutions and the nature of the tuberculous process in the lung.

Two controlled groups were studied. One was a group of 161 female nurses in training and the other was a group of 46 tuberculous women.

Measured dilutions of 1:100,000, 1:20,000, 1:1,000 and 1:100 were used throughout. In addition to the tuberculin tests a thorough history, general physical examination and repeated stereoscopic x-ray examination of the lungs were obtained in every case.

All individuals were under observation for from three months to two years.

The individual response to measured dilutions of tuberculin bore no consistent relation to the activity or inactivity of the tuberculous process.

ENCEPHALITIS, Epidemic, Epidemiology of, St. Louis Type, Leake, J. P., Musson, E. K., and Chope, H. D. J. A. M. A. **103**: 728, 1934.

The type of disease in the St. Louis outbreak was unlike that in the sporadic cases of the Conomo disease but very much like Type B of the Japanese outbreak in 1924, and almost exactly like the Paris, Ill., outbreak of 1933.

The cases were fairly accurately and completely reported.

The case rate for the entire area was 100 per hundred thousand—69 per hundred thousand for the city and 212 per hundred thousand for the county.

There was no predilection by sex or color.

There was a striking increase in both incidence and fatality rates with age.

The fatality rate was higher in the city than in the county.

The incubation period in different cases showed a variation between nine and fourteen days, with possibly wider limits.

There was a notable rarity of multiple cases in the same family and of obvious contagion between cases.

Between communities the spread was obviously by human contagion; but as regards individuals, individual susceptibility, in which age played a part, appears to be more important than contagion.

The disease appears to be limited seasonally in its typical form.

Water supply and milk supply were eliminated as possible mediums of transmission.

Entomologic experiments with the mosquito as a possible vector were negative.

GASTRIC ANALYSIS, Detection of Free Acid in Patients With Suspected Anacidity, Necheles, H., and Scheman, L. J. A. M. A. **103**: 107, 1934.

The authors comment that free HCl will combine quickly with mucus thus leading, under the usual methods of gastric analysis, to reports of its absence even though actually it may be present in small amounts associated with an excess of mucus.

The apparatus described below permits a determination of acidity as soon as the sample passes out of the stomach.

It consists of a glass T-tube, the vertical arm of which is wide enough to hold a stopper with a bore. The lower end of a buret is used, the glass tube below the stopcock being pushed through the bore of the stopper. The opening of this glass tube is made as narrow as possible. One end of the T-piece is connected to a suction device with a mercury manometer and needle wave. The other end of the T-tube is connected to the stomach tube. The container above the stopcock is filled with Topfer's reagent. Small sealed glass tubes with various concentrations of hydrochloric acid and Topfer's reagent may conveniently be attached alongside the horizontal part of the T-tube to read the concentration of free acid in the gastric juice. The base of the wooden stand on which the T-tube rests is painted white. As the suction works and gastric contents appear in the T-tube, the stopcock is opened, permitting a drop of reagent to come in contact with the stomach juice, and notes are taken of the color reaction. Mucus, bile and pure stomach juice can thus be tested separately before neutralization takes place.

ANEROBES, Method for Plate Cultures of, Trambusti, B. Diagn. E. Techn. di Lab. **5**: 23, 1934.

The method following permits the isolation of anerobes on plates and examination of the colonies without destruction of the surface of the medium.

Three pieces of glass rod or tubing (sealed at the ends) are cut of a length slightly less than the diameter of the Petri dish to be used and arranged on the inverted lid in the form of an equilateral triangle. The medium is added and then the inverted bottom of the dish placed upon the glass triangle. The space between the lid and bottom is then sealed as usual and incubated.

GONORRHEA, Treatment of Acute by Means of a New Gonococcic Vaccine of Low Toxicity, Price, I. N. O., and King, A. J. Brit. M. J. **1**: 748, 1934.

The authors report favorably upon a gonococci vaccine prepared as follows:

A forty-eight-hour growth of gonococci (hydrocele agar, pH 7.5, in a triangular Roux bottle incubated at 37.5° C.) is washed off into a cylinder with 100 c.c. of physiological salt solution, giving a suspension containing about 180 million organisms per cubic centimeter.

After the addition of 1 c.c. of N/10 NaOH the cylinder is placed in a 37.5° C. water-bath for two hours to dissolve the gonococci. The fluid is then filtered through sterile lint, 1.5 c.c. of N/10 HCl added. After twenty or thirty minutes the white floccules

appearing are removed by centrifuging (3,000 r. p. m.) and suspended in 9 c.c. of sterile normal saline. To this suspension N/10 NaOH is added drop by drop until the flocculcs go into solution (pH 7.5). When this occurs 1 c.c. of 1 per cent formaldehyde in normal saline is added.

This solution constitutes the vaccine, 1 c.c. containing the protein of approximately 180 million organisms.

Subcutaneous injections are given daily into the gluteal muscles, starting with 0.25 c.c. and increasing to a maximum of 1.5 c.c. according to the reaction produced.

The vaccine is most effective in chronic gonorrheal conditions but appeared to lessen the incidence of complications when given during the acute stages.

CYANIDE POISONING, Gettler, A. O., and St. George, A. J. Am. J. Clin. Path. 4: 429, 1934.

The authors recommend the following procedures in the study of cyanide poisoning:

If the poison has been taken by mouth, the stomach contents and brain should be analyzed. Analysis of the brain is necessary for the purpose of ruling out the possibility of the poison having been introduced into the stomach after death. If the poisoning resulted from inhalation, the lungs and brain must be examined. In cases of poisoning by inhalation, usually none, or only the very faintest trace, is found in the stomach contents. This is of tremendous importance from the medicolegal aspect.

METHOD OF ISOLATION OF CYANIDE

The tissues are cooled by keeping them in an ice box. Two hundred to 500 gm. of tissue are ground up. Care should be taken to keep the tissue cold since hydrocyanic acid may volatilize if warm. If stomach contents are analyzed, usually one-fifth of the total volume of the contents is used. The ground-up tissue or the stomach contents are placed in a one liter flask and acidified with tartaric acid. The material is then distilled with steam, using a well-cooled condenser the tip of which is bent to serve as an adapter and dipped into 5 c.c. of 5 per cent sodium hydroxide solution in a receiving flask. The latter should be packed in ice. One hundred cubic centimeters of distillate are collected, which is ample to recover all the cyanide present, the following tests being employed:

QUALITATIVE TESTS

1. *Schonbein's Test.*—Suspend a strip of filter paper, impregnated with guaiac and copper sulphate, over the material in a flask, the paper being held in place by the stopper. (Dip strip of filter paper into a freshly prepared alcoholic solution of guaiac 1:10; then let dry; when dry, moisten it with dilute (1:10,000) copper sulphate solution.) If color does not change, cyanide is absent and no further tests need be made. If a blue color results, cyanide may be present. The test is very sensitive but not specific; hydrochloric acid, nitric acid, chlorine, bromine, ozone, hydrogen peroxide, as well as some other substances also give a positive test. This test may be used as a preliminary one at the necropsy table. The following two tests must be employed since they are specific for cyanide:

2. *Prussian Blue Test.*—To 5 c.c. of distillate, add 3 c.c. of 25 per cent sodium hydroxide, then a few drops of freshly prepared ferrous sulphate solution and a few drops of ferric chloride solution. Warm a little. Let cool and add concentrated hydrochloric acid, dropwise, until the dirty brown precipitate just dissolves; avoid excess hydrochloric acid. If cyanide is present, a deep blue precipitate (Prussian blue) appears. If only a trace of cyanide is present, a green solution results instead of a blue precipitate, but, on standing several hours, a small flocculent Prussian blue precipitate settles (sensitive to one part in 50,000).

3. *Liebig's Test.*—To 10 c.c. of distillate, add 1 c.c. of yellow ammonium sulphide and evaporate to dryness on the water-bath. When dry, add 5 c.c. of 5 per cent hydrochloric acid solution, warm a little and stir well to dissolve all of the thiocyanate that was formed

during the evaporation. Let stand two hours, then filter. To the filtrate, add 5 to 10 drops of 10 per cent ferric chloride solution. If cyanide is present, a deep red color results (sensitive to one part in 10 million).

The following tests may be used, but they are not specific for cyanide:

4. *Vortmann's Test.*—To 5 c.c. of distillate, add a few drops of potassium nitrite solution, then 2 to 4 drops of ferric chloride solution and then enough dilute sulphuric acid until the color of the solution becomes a bright yellow. The solution should then be boiled, after which cool and add ammonium hydroxide until all of the iron is precipitated. Filter off the precipitate. To the filtrate add a few drops of a very dilute solution of ammonium sulphide. If cyanide is present, a play of colors results, violet, blue, green, yellow.

5. *Picric Acid Test.*—To 5 c.c. of distillate (slightly alkaline) add a few drops of picric acid solution and warm gently. If cyanide is present, a red color develops (sensitive to one part in one million).

6. *Phenolphthalin Test.*—To 5 c.c. of distillate, add a few drops of alkaline phenolphthalin solution (reduced phenolphthalein), then a few drops of 1:2,000 copper sulphate solution. If cyanide is present, a red color develops (sensitive to one part in 20 million).

7. *Silver Test.*—To 2 c.c. of distillate, add nitric acid until reaction is acid, then add a few drops of silver nitrate solution. If cyanide is present, a white precipitate of silver cyanide results.

QUANTITATIVE TEST

For quantitative analysis, a weighed amount of tissue is distilled, as described in the qualitative procedure. In the receiving flask, however, instead of having dilute sodium hydroxide, 20 c.c. of 10 per cent silver nitrate solution are used, acidified with nitric acid. In order to make certain that all of the cyanide is isolated, distillation is continued until 200 c.c. of distillate are obtained. During the distillation, the cyanide precipitates as silver cyanide. This is then filtered through a previously weighed Gooch crucible, washed, dried and weighed. From the weight of the silver cyanide, the amount of cyanide in the material analyzed is calculated as HCN.

In quantitative analysis, if the poison was taken by mouth, the entire amount of cyanide is determined in the gastrointestinal tract. This is then multiplied by 100/98, which gives the amount in the entire body. This fraction is used because approximately 98 per cent of the cyanide, if taken by ingestion, remains within the stomach contents. If the poison was introduced by inhalation or injection, parts of all the organs and tissues are analyzed and, from these results, the amount present in the entire body is calculated.

The lethal dose of cyanide is accepted as 50 mg., calculated as hydrocyanic acid.

The following factors interfere with the determination of the presence of cyanide:

1. Traces of hydrogen cyanide are produced during the first days of putrefaction, but this disappears in the later stages.

2. Cyanides present in the tissues disappear during prolonged putrefaction and are changed to sulphocyanide.

3. In the stomach contents, where the bulk of the cyanide remains at death, putrefaction is of little importance.

4. Embalming with formaldehyde interferes greatly in the tests, the cyanide forming condensation products with the formaldehyde.

SEDIMENTATION TESTS, Repeated. Van Antwerp, L. D. Am. J. Dis. Child. **48**: 814, 1934.

The observation that sedimentation rates in children show an unexplained tendency to vary widely without intervening clinical causes should be of value to pediatricians, regardless of the cause. That this occurs commonly is demonstrated by the fact that 27 per cent of the author's patients had readings erroneous enough to mislead in their interpretation. His experience, however, shows that a repeated test will usually give the correct reading. Occasionally a repeated test will show a persistently high rate, though this is not common. In

such an event, careful clinical search should satisfy one as to the presence or absence of any condition warranting a high rate. A persistently high rate need serve, therefore, only as a warning of the possibility of the presence of a pathologic condition; it need not be held as conclusive.

Two sedimentation tests are done on successive days on all newly admitted patients, the lower rate being taken as the one reflecting the presence or absence of a destructive lesion. Van Antwerp is being forced to the conclusion that apprehension on the part of the patient is a factor in erroneous results in sedimentation tests, though he has not thus far been able to prove it. If such is the case, it will be necessary to repeat tests on patients giving a high rate as basal metabolic tests are now repeated. It is already well known that certain types of insanity are characterized by high rates of sedimentation and it is interesting to consider the possibility that the factor influencing the rate in this class of patients may be that of apprehension, because of emotional instability.

Later observations comparing blood sugar content with rates of sedimentation will be offered as a factor of proof or disproof of the contention that apprehension influences the rate of sedimentation.

EOSINOPHILIA, Rapid Method for Determination of, Freedman, T. B. J. A. M. A. 103: 1618, 1934.

In place of the usual acetic acid-diluting fluid, the following fluid, which is the modification of Camara and Alvarez of that described by von Domarus, is employed:

Aqueous eosin 1 per cent	5 c.c.
Acetone	5 c.c.
Distilled water	100 c.c.

The count is made in the usual manner in the ordinary counting chamber. This diluting fluid stains only the eosinophilic cells and they are easily distinguishable with the high dry power of the microscope. The unstained leucocytes appear as gray bodies in this diluting fluid.

It is advisable to fill also a pipette with the ordinary acetic acid-diluting fluid and use this count as a check until one has had some experience with the fluid described.

CHORIONEPITHELIOMA, Early Diagnosis By Quantitative Determinations of Pituitary-Like Principle From the Urine of Pregnancy, Leventhal, M. L., and Saphir, W. J. A. M. A. **103:** 668, 1934.

The quantitative determination of the gonadotropic substance in the urine is an important biologic test in the early diagnosis of chorionepithelioma.

Following the expulsion of a hydatidiform mole, this quantitative test forms a prognostic index for chorionepithelioma formation.

In the absence of clinical manifestations, an amount of gonadotropic substance in the urine in excess of 20,000 mouse units per liter indicates an early chorionepithelioma.

A case of early chorionepithelioma was diagnosed and an operation was performed solely on the basis of the laboratory finding of 333,000 mouse units of gonadotropic substance per liter of urine in a patient who had expelled a hydatid mole four and one-half months previously.

Recurrence of chorionepithelioma or metastases may be discovered by the Aschheim-Zondek reaction, and this should determine the use of radiation therapy.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Definite Diagnosis In General Practice*

AS DR. MUSSER remarks in the foreword to this book, "there is no quick road to diagnosis," and the importance of diagnosis in the management of disease requires no comment.

In constructing his book Dr. Kitchens has chosen, from among the diseases to which the human race is heir, 407, representing those most likely to be encountered in the most wide and varied practice; and from his own experience as well as from a thorough study of medical literature, has selected 506 symptoms of definite diagnostic importance.

The book is planned for use as a quick reference, as a simplified means of differential diagnosis, and as a method assisting in selective or eliminative diagnosis, or, as often called, diagnosis by exclusion.

The volume is divided into two sections.

Part I includes, first, a Symptom Index which presents in alphabetical order 506 symptoms of 407 diseases, each symptom being preceded by the page number on which that symptom is found.

Following the Symptom Index each symptom is presented on a separate page and under each symptom are listed those diseases in which that symptom is of important diagnostic significance. Furthermore, each disease is preceded by the page number in Part II on which the full symptomatology of that disease is found.

Part II presents, first, 506 diseases, one to a page, under each of which are presented those symptoms of diagnostic significance, giving first Subjective Symptoms, then Objective Symptoms, and finally Laboratory Findings. Each symptom is preceded by the page number in Part I where that symptom is listed.

Following the presentation of diseases, there is a Disease Index listing in alphabetical order the 506 diseases, each disease being preceded by the page number in Part II where the full symptomatology of that disease is found.

If it is desired to review quickly the symptomatology of any disease, the reader turns to the disease in Part II. Or, given a symptom, if it is desired to learn at a glance those diseases in which that symptom is found, the reader turns to the symptom in Part I.

To differentiate quickly any two or more diseases, the reader turns to those diseases in Part II and checks the complete and possible symptomatology of one against the other.

The book provides ample space for the addition by the reader of observations reflecting his own experience or the observations of others so that it may be kept continually up to date.

The book is simple in its method of use and should prove without doubt, as it is intended to be, of definite aid in the formation of a diagnosis.

Statistical Methods for Research Workers†

THIS volume is one of a series of Biological Monographs and Manuals issued under the editorship of F. A. E. Crew.

The purpose of the present volume is to present to research workers the practical procedures relative to statistical methods which are appropriate to their problems and for the presentation of their results.

That the book has reached its fifth edition testifies to its usefulness.

*Definite Diagnosis In General Practice. By W. K. Kitchens, M.D. Cloth, pp. 1000. W. B. Saunders Co., Philadelphia, Pa.

†Statistical Methods for Research Workers. By R. A. Fisher, S.C. Formerly Fellow of Gonville and Cains College, Cambridge, etc. Cloth, ed. 5, pp. 319, 6 insert tables, 68 text tables. Oliver and Boyd, Edinburgh.

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EDITORIALS

Etiology of Granulopenia (Agranulocytosis)

EVER since Schultz[1] in 1922 described as a clinical entity the association of gangrenous throat lesions with extreme leucopenia, there has been an increasing interest in the condition and much speculation concerning its etiology. It is now known that the primary change is found in the bone marrow and that the granulopenia is only a reflection of the marrow dysfunction. The necrotic local lesions result in turn from the loss of the defense normally provided by the neutrophilic leucocytes. Often there is no superficial necrosis even with a marked leucopenia, although weakness is a very constant symptom, and it is usually accompanied by chills and fever.

In his cases, Schultz found a marked aplasia of the myeloid cells in the bone marrow with little involvement of erythrocytes or platelets. Further studies of the marrow of patients dying from granulopenia with or without mucosal lesions have revealed widely varying findings. Often the marrow is

hyperplastic rather than aplastic, but there is always some disturbance in the state or rhythm of development of the granulocytes. Fitz-Hugh and Krumbhaar[2] emphasize the lack of maturation of the neutrophiles even when there is an abundant supply of immature myeloid cells, and they suggest that the disease is fundamentally a "maturation arrest" of the granulocytes. Jaffé[3] found striking qualitative changes in the myelocytes in the marrow which were evidenced by marked degenerative changes. It is apparent that the marrow picture must vary with the stage of the disease. In the early stage, there may be only a disturbance in maturation; later, the myelocytes show evidence of an intoxication; and still later, the myeloid tissue may be aplastic. With massive dosage of the agent responsible for the marrow damage or a hypersusceptibility of the patient to small doses, extreme marrow degeneration may occur early in the course of the disease. Thus, it is apparent that the marrow picture is the primary and important one. The change is essentially a myelotoxicosis which varies greatly in degree and intensity. At times the clinical picture of the disease is seen with some known disorder of the marrow, such as aplastic anemia, leucemia, or myeloma. Here the cause of the clinical picture is the same as that responsible for the cryptic cases, although the bone marrow disturbance is different from that seen in idiopathic granulopenia.

The most perplexing problem has been the etiology of the marrow injury. Radiant energy may cause a marked depression of the marrow, but this can usually be ruled out as a possible causative factor. Overwhelming infection may affect the marrow in a similar manner and cause a granulopenia. However, such an infection usually is apparent as the primary disease. The local lesions of granulopenia show a great variety of organisms and the blood culture is often positive late in the disease. No organism, however, has been proved to be constantly and etiologically related to the disease. It is possible that a relatively mild infection may further depress a hypoplastic marrow and produce a granulopenia.

The possibility of a chemical cause for the disease has always been intriguing. It has long been known that certain chemicals will cause an aplasia of the bone marrow with involvement of all marrow elements. Benzene is the classic example of such a drug. It is interesting that most other drugs which have a similar action, such as arsphenamine, also contain the benzene ring. This has been emphasized by Kracke.[4] In granulopenia we have a marrow disturbance similar to that caused by benzene except that there is selective involvement of myeloid tissue rather than simultaneous involvement of myeloid, erythroblastic and platelet-forming tissues. It is true, however, that in granulopenia there is usually some involvement of red cells and platelets, although in a much less degree than in aplastic anemia. A most important contribution to the etiology of the disease is the recent demonstration that amidopyrine and similar commonly used drugs may produce the disease. Kracke[4] noted that eight of nine patients with granulopenia of undetermined origin had been taking drugs of the coal tar series prior to the onset of symptoms. However, he was unable to affect the leucocyte count experimentally with such drugs, although he was able to do so with the oxidation products of benzene. Madison and Squier[5]

were the first to offer convincing proof, largely from clinical observation, that amidopyrine alone or in combination is a definite cause of granulopenia. Two of their patients, after recovering from an attack, again developed a profound granulopenia when a single dose of amidopyrine was taken. Sturgis and Isaacs[6] report similar observations. Evidence is rapidly accumulating[7, 8, 9] that amidopyrine and similar drugs are the most important etiologic agents in idiopathic granulopenia. Kracke[10] in reviewing the relationship of drugs to the etiology of the disease concludes that drugs containing the benzamine group (amidopyrine, phenacetin, acetanilid, arsphenamine, and neoarsphenamine) are most likely to cause the disease.

The drugs which have been so incriminated are very commonly used. The question arises why they relatively seldom cause trouble. Pepper[11] has pointed out the frequency of allergy and drug sensitization in cases of granulopenia. This is a most important observation because it seems that, in most cases, the sensitivity of the patient determines the toxic effect of a small dose of a drug which has a specific affinity for myeloid tissues. This also well explains the difficulty in reproducing the disease experimentally. In some cases the disease is due to large doses over a long period of time with cumulative action or development of sensitivity by reason of prolonged use. At times the marrow may be already hypoplastic making it more susceptible to further injury.

The etiology of granulopenia thus seems solved in the demonstration of drug relationship to the disease. Time will probably prove that most cases of so-called idiopathic granulopenia are due to the toxic action of some drug on the marrow either by reason of the hypersensitivity of the patient or overdosage of a drug with a selective action on normal or hypoplastic myeloid tissue.

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—*R. L. H.*